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Effects of Leaflet Orientation and Root Morphology on Physiological Traits and Yield in Soybeans.

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I am submitting herewith a dissertation written by Richard DeWayne Johnson entitled "Effects of Leaflet Orientation and Root Morphology on Physiological Traits and Yield in Soybeans.." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

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Effects of Leaflet Orientation and Root Morphology on Physiological Traits and Yield in Soybeans

A Dissertation

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Degree

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Richard DeWayne Johnson

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Dedication

This thesis is dedicated to my Grandparents Millard and Edna Johnson, who planted the seed of God's love through their faith and their works. Much of what I am I owe to their love and example in living godly lives. I give them the roses of this tribute for raising me to love and respect God, family, and work, and to treat others as you would wish to be treated. God heard the prayers of a child orphaned by divorce and blessed him with new parents whose love and kindness abounded beyond measure.

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Abstract

Drought is the most important abiotic stress adversely affecting soybean (*Glycine max* (L.) Merr.) yield. Leaflet orientation has been shown to reduce leaflet temperatures and transpiration while root morphology has been related to slower wilting phenotypes. The objective of this study was to investigate effects of leaflet orientation and rooting morphology on whole plant transpiration, yield, water use efficiency, and other physiological traits in soybeans using grafting techniques, population lines, near-isogenic lines, and restrained leaf canopy experiments. Experiments were conducted in Knoxville, TN with additional yield trial plots at Springfield, Spring Hill, and Milan, TN. Data were collected on whole plant transpiration, seed yield, leaflet orientation phenotype, root morphology, PAR, SAR, maturity, height, lodging, biomass accumulation, leaf area, photosynthesis, canopy light penetration, seed size, seed protein and oil. Grafting experiments revealed that plant shoots affected many of the measured traits but did not condition root phenotype. Root effects on measured traits were not significant. Effects of scion and root morphologies on measured traits could not be separated from genetic differences of the lines grafted. Population line analysis found no clear association between leaflet orientation and transpiration or yield. Leaflet orientation associated with some traits but those associations reflected the phenotype of parental lines, suggesting genetic linkage. Orienting leaves had cooler temperatures relative to leaves receiving direct sunlight. High orienting lines allowed more sunlight penetration into lower canopy which had a positive effect on mid-canopy photosynthesis. Leaflet orientation correlated with root morphology. Overall, root morphology had little effect on measured traits. Leaflet orientation and root morphology frequency distributions approximated normal distributions suggesting traits are polygenic.

Experiments with near-isogenic lines detected no consistent patterns or significant effects due to differing leaflet orientation and root morphology on measured traits. This may have been due to lack of prominent differences in leaflet and root phenotypes between isogenic line pairs. Restrained canopy evaluations revealed no statistical differences in whole plant transpiration rates between plants allowed to orient leaves versus those with leaflets restrained. Further research is needed to investigate the effects of leaflet orientation and root morphology on yield and other physiological characteristics in soybean.

Preface

The world-wide importance of soybeans (*Glycine max* (L.) Merr.) as a crop has increased steadily over the past 30 years. Not only is soybean the world's primary source of protein and oil but it is often referred to as a miracle crop due to its more than 200 uses in feed, food and industrial applications (Pathan et al., 2007). The increased importance of soybean as a world crop has led to a sizable expansion in world soybean production from 70 million tons in 1984 to 251 million tons in 2011 (www.soystats.com). Improvement of soybean cultivars for higher yield and resistance to biotic and abiotic stresses is therefore of great interest to plant breeders, producers, manufacturers and consumers.

Drought tolerance

Research into plant responses to water stress is becoming increasingly important, as most climate change scenarios suggest an increase in arid land area in many regions of the world. On a global basis, drought in conjunction with high temperatures and solar radiation constitutes the most important environmental factors limiting crop productivity. Agriculture is a major consumer of water resources in many regions of the world. As the human population increases, water will become a scarcer commodity. A better understanding of drought tolerance in plants is vital for improved management practices and breeding strategies (Chaves et al., 2003).

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress that adversely affects soybean seed quality and reduces yield by approximately 40%. Consequently, drought tolerance is a highly sought after trait in soybean cultivars. Drought tolerance is a complex response and is conditioned by the interaction of several genetic traits of the plant to environmental conditions (Chaves et al., 2003; Pathan et al., 2007). Knowledge of these trait processes is needed not only to understand plant resistance to drought stress but also to improve crop management and breeding techniques. Many of the traits that are attributed to plant adaptation during drought such as phenology, root size and depth, and hydraulic conductivity are associated with plant development and structure and are constitutive rather than stress induced. A considerable part of plant resistance to drought is the ability to dissipate or avoid excess radiation. The nature of the mechanisms responsible for leaf photoprotection, especially those related to thermal dissipation and oxidative stress are therefore of great interest. A desirable plant type would be one that could endure drought conditions while maintaining a higher level of productivity by avoiding tissue dehydration, maintaining tissue water potential and photosynthesis as high as possible. Adaptive traits which condition dehydration avoidance include those which minimize excessive water loss and maximize water uptake. Water loss can be reduced by reducing light absorbance via steep leaf angles. Water uptake can be maximized by increasing the rooting volume and/or depth (Chaves et al., 2003). Two potential traits of interest are therefore leaflet orientation and root morphology. Leaflet orientation addresses the need to reduce water loss and root morphology addresses the ability to maximize water uptake.

Leaflet orientation

Many species of plants are capable of leaf movements in response to external stimuli (Ehleringer and Forseth, 1980). Leaf movement in response to light, known as heliotropism, can be classified as either diaheliotropic (light seeking) or paraheliotropic (light avoiding). Plants exhibiting diaheliotropism orient the plane of the leaf blade perpendicular to incident light rays, while plants exhibiting paraheliotropism orient the plane of the leaf blade parallel to incident light rays. Soybean exhibits both diaheliotropic and paraheliotropic movements, with the degree of movement being dependent on genotypic response (Wofford and Allen, 1982) and various levels of environmental stimuli (Ehleringer and Forseth, 1989; Rosa and Forseth, 1995).

Pulvini

The structure that facilitates heliotropism in soybean is the pulvinus. The pulvinus is a joint-like structure occurring at the base of leaflets which facilitates reversible leaflet movements in a relatively short amount of time. Schwartz and Koller (1978) found that the leaves of *Lavatera certica* moved in conjunction with potassium (K^+) uptake and release. They theorized that under high light levels, the pulvini turgor increases when K^+ uptake is activated. Reduced light levels cause pulvini turgor to decrease by deactivating K^+ uptake with a consequential leakage of K^+ from the pulvini. Wofford and Allen (1982) found that K^+ concentration in soybean pulvini was significantly higher when leaves were exhibiting photonastic movement than during non-orientation. They surmised that K^+ concentration in the pulvinus may have a role in regulating leaf orientation movements, perhaps by inducing changes in turgor pressure. Oosterhuis et al. (1985), in their study of soybean leaflet movements, also concluded that soybean leaflet movements were possibly controlled by osmotic and pressure potentials, and K^+ concentration changes across the pulvinus membrane. Schwartz et al. (1987) found that the perception of photonastic excitation in *Melilotus indicus* (Fabaceae) was located in the pulvinus. In order to begin active transport of K^+, the pulvinus must receive external light stimulation, and although the entire pulvinus is sensitive to light, the abaxial surface is more sensitive than the adaxial surface. Donahue and Berg (1990) studied the soybean pulvinus as the structure responsible for leaflet movement in soybean, and found that it is sensitive only to blue light irradiance. Homologues of soybean genes have been found to control light regulated leaf movements in the legume species *Samanea saman* (Moshelion et al., 2002). These genes are thought to control the action of K^+ proton pumps in the pulvini. These proton pumps may be triggered by blue light irradiance as Okazaki (2002) found to be the case in *Phaseolus vulgaris.*

Discovering which environmental factors influence heliotropism aids in understanding the causes and effects of leaf movements. Kao and Forseth (1993) reported that paraheliotropic movements in soybeans are affected by available nitrogen, air temperature, photosynthetic photon flux, and soil water potential. Researchers also found that leaf angles measured on leaves of drought stressed soybean plants were steeper (more paraheliotropic) relative to those of well-watered plants (Oosterhuis et al., 1985; Rosa et al., 1991; Kao and Forseth 1992a,b). Beilenber et al. (2003) also found that temperature influences leaflet orientation in *Phaselous* species.

Paraheliotropism and diaheliotropism provide a means by which the plant can alter the arrangement of its leaves in order to gain maximum benefit from the environment. Advantages of changing leaf angle and light absorbance include increased total <u>canopy light interception</u> (Kawashima, 1969 a,b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000), <u>increased photosynthetic efficiency</u> (Pichard and Forseth, 1988; Gamon and Pearcy, 1989; He et al., 1996; Kawashima, 1969a,b; Arena et al., 2008), and, <u>increased yield</u> (Wang et al., 1995; Chang and Tagumpay, 1970; Mickelson et al., 2002; Pendleton et al., 1968; Pepper et al., 1977). Leaflet orientation can also <u>reduce leaf temperature</u> (Pichard and Forseth, 1988; Gamon and Pearcy, 1989; Wang et al., 1993; Forseth and Teramura, 1986; Rosa et al., 1991; Kao and Forseth, 1992a; Paris, 1997; Bielenberg et al., 2003; Yu and Berg, 1994; Rosa and Forseth, 1995;

Isoda and Wang, 2002; Arena et al., 2008; He et al., 1996; Stevenson and Shaw, 1971; Isoda et al., 1992, 1993; Isoda and Tomagae, 2003) which can <u>reduce excessive transpiration rates</u> (Pichard and Forseth, 1988; Bielenberg et al., 2003; Kao and Forseth, 1992a; Yu and Berg, 1994; Isoda and Wang, 2001, 2002; Wien and Wallace 1973; Shackel and Hall, 1979; Meyer and Walker, 1981; Berg and Hsiao, 1986; Forseth and Teramura, 1986; Berg and Heuchelin, 1990). Additionally paraheliotropism can <u>reduce photoinhibition</u> (Hirata et al., 1983; Prichard and Forseth, 1988;; Rosa et al., 1991; Rosa and Forseth, 1995; He et al., 1996; Jiang et al., 2006; Kao and Tsai, 1998), and <u>increase water use efficiencies</u> (Prichard and Forseth, 1988; Rosa et al., 1991; Kao and Forseth, 1992a; Bielenberg et al., 2003; Kao and Tsai, 1998).

Leaflet orientation and water use

Paraheliotropism (light avoiding movement) has been observed in many plant species as a means of reducing moisture stress (Wien and Wallace, 1973; Shackel and Hall, 1979, Ehleringer and Forseth, 1980; Meyer and Walker, 1981; Berg and Hsiao, 1986; Forseth and Teramura, 1986; Gamon and Pearcey, 1989; Berg and Heuchelin, 1990; Donahue and Berg, 1990). In soybean, this phenomenon may be a mechanism to reduce water loss while maintaining some level of productivity as reported by Meyer and Walker (1981). Paraheliotropic leaf movements reduce transpirational water loss by lowering light interception of leaves, consequently improving water status and lowering leaf temperature. Ehleringer and Forseth (1980) similarly reported that during drought stress, leaves minimize absorption of solar radiation, consequently reducing the heat load on the leaf by decreasing leaf temperature and transpiration rate. This phenomenon, in conjunction with stomatal closure, reduces transpiration under conditions of low water and high incident light (Berg and Heuchelin, 1990). Stevenson and Shaw (1971) reported that soybean leaf temperature was lower for leaves exhibiting paraheliotropism, and higher for leaves exhibiting diaheliotropism (light seeking movement). Based on their data, they suggested that less leaf resistance to water vapor diffusion and lower leaf temperatures would occur in soybean canopies with upright leaves. They further suggested that incorporating such attributes into a breeding program would be useful in developing soybean cultivars with tolerance to moisture stress. However, as Costa and Ariyawansha (1997), and Zhanbin (1997) caution, extensive testing should be carried out before selection since cultivars may perform differently under differing levels of moisture stress and other environmental conditions. Forseth and Teramura (1986) reported that paraheliotropism in kudzu (*Pueraria montana*) leaves reduced leaf irradiance at midday by one-half, leaf temperatures by 5-6ºC, and transpiration loss by 18-26% when compared to fixed, horizontal leaves. They speculated that this improved the water use efficiency and helped to avoid thermal and photo inhibitor damage to the photosynthetic apparatus. Similar conclusions were found by Raeini-Sarjaz and Chalavi (2008) in studies involving *Phaseolus vulgaris* L. In their reviews Ehleringer and Comstock (1987) and Ehleringer and Forseth (1987) relate that leaflet orientation protects leaf tissues from excessive irradiance, lowering leaflet temperatures, excessive water loss and resulting in an increase in water use efficiency.

Research conducted at the University of Tennessee demonstrated that soybean cultivars differ in their ability to orient leaflets during the course of the day (Wofford and Allen, 1982). Most cultivars exhibit high leaflet orientation (paraheliotropism) and move their leaves during the course of the day such that the leaves have maximum exposure to the sun in the early and late parts of the day, but during mid-day the leaves are oriented parallel to sunlight such that the surface of the leaves has minimum exposure to the sun. A lesser number of cultivars exhibit low leaflet orientation where the leaf surface remains relatively flat and changes little relative to the position and intensity of sunlight, even during the mid-day period of highest irradiance. These "low leaflet orienting" types are therefore relatively less paraheliotropic. In a study of the cultivar Essex (high leaflet orientation) and Dare (low leaflet orientation), the two cultivars produced about equal yields; however Essex used about one-half the amount of water as Dare during the growing season (Paris, 1997). Similarly Beilenber et al. (2003) found that leaflet orientation in *Phaseolus* resulted in lower leaflet temperatures and transpirational water loss while maintaining photosynthesis rates thereby increasing water use efficiencies.

Light interception, leaflet temperature, and photosynthesis

Increased exposure to solar radiation increases leaf surface temperature and water loss (Gates, 1962). Orienting leaves parallel to the sun can decrease the leaf temperature and transpiration rate therefore allowing plants to be well adapted to drought conditions (Ehleringer and Forseth, 1980).

The efficiency at which solar radiation is transformed into biomass and the amount of radiation available are among the most important of the numerous factors affecting crop yield (Russell et al., 1989). However, light over-saturation of photosynthesis leads to a decline in radiation-conversion efficiency. For instance, in rice (*Oryza sativa*), this was estimated to be approximately 17%, depending on cultivar and growing conditions (Murata and Matsushima, 1975). There are many different factors influencing leaf photosynthesis rates in response to light levels. These include elevated leaf temperatures that accompany high irradiance causing metabolic imbalances, enzymatic activity changes and deleterious effects on thylakoid function (Pastenes and Horton, 1996a,b), enhanced photoinhibition (Fuse et al., 1993), and enhanced photorespiration (Leegood and Edwards, 1996). Additionally, leaf angle has been identified as influencing the degree of light saturation of upper leaves, lower canopy leaves and overall photosynthetic rates in rice (Yoshida, 1981; Murchie et al., 1999).

In work with soybean, Lugg and Sinclair (1981) found that upper leaflets of the canopy maintained a higher net photosynthetic rate per unit leaf area than did the lower leaflets. This seemed to be mostly due to shading as the lower leaves were found to have photosynthetic rates similar to upper canopy leaves when unshaded. Kawashima (1969 a,b) found that soybean leaflets exhibiting paraheliotropism in the upper canopy allowed light to penetrate more deeply into the canopy, increasing photosynthetic output of the lower leaves, thus allowing total photosynthetic efficiency of the plant to be improved. Vertical leaf angles decrease the amount of solar radiation intercepted by the leaf. However photosynthetic rate response in plants to solar radiation is nonlinear and saturates below the intensity of direct ambient sunlight (van Zanten et al., 2010). Soybeans are reported to maximize their photosynthetic rates at less than one-third the amount of full sunlight according to Beuerlein and Pendleton (1971). Vertical leaflet orientation increases overall photosynthesis by allowing the upper canopy leaves to continue to photosynthesize under lower than ambient sunlight while also allowing lower canopy leaves to contribute at an increased rate (van Zanten et al., 2010). Pearce et al. (1967) found that net photosynthesis in barley (*Hordeum vulgare*), was increased by more vertical leaf angles as this morphology allowed more light to penetrate into the lower canopy. Similarly, Wien and Wallace (1973) found that upper leaves of *Phaseolus vulgaris* exhibited paraheliotropism, allowing light from above to penetrate more deeply into the canopy, while lower leaves exhibited diaheliotropism in response to incoming light from the upper canopy.

Kao and Tsai (1998) studied leaf movements in three soybean species and found that paraheliotropism seemed to enhance water use efficiency and decrease the risk of photoinhibition in plants under water stress. Grant (1999) found that soybean plants that exhibit paraheliotropism are able to reduce UV-B irradiance in contrast to plants that do not orient leaflets. Ikeda and Matsuda (2002) studied photosynthetic efficiency differences in soybean leaves which were restrained from orienting versus naturally orienting. Their results indicated that paraheliotropic leaflet movements are an adaptation which optimizes net leaflet photosynthesis. Arena et al. (2008) compared photosynthetic performance differences of black locust (*Robinia pseudoacacia* L.) leaves which normally exhibit paraheliotropism. Leaves which were restrained from orienting received more sunlight and were found to have higher temperatures, decreased photosynthesis, and decreased stomatal conductance. Leaflet orientation was found to avoid photoinhibition resulting in photosynthetic increase for the plant. Other researchers have found that leaflet orientation lowers the leaflet temperatures (Wang et al., 1993; Paris, 1997;), lower transpiration rates (Isoda and Wang 2001), increases light penetration into the lower canopy allowing the plant to maintain a higher overall photosynthetic rate (Wang et al.,1994; Reynolds et al., 2000, van Zanten et al., 2010) which may increase yield (Kawashima, 1969 a,b; Wang et al., 1995)

Isoda and Wang (2002) studied leaf temperature and transpiration rates of cotton (*Gossypium hirsutum*) versus soybeans and found that soybeans were able to reduce leaf temperatures and transpiration rates. This was attributed to the soybean cultivars ability orient its leaves in a paraheliotropic manner. In a study involving restrained and unrestrained soybean leaflets, Isoda et al. (1992, 1993) found that the paraheliotropic movements of soybean leaflets regulate light interception and leaf temperature. Isoda and Tomagae (2003) found differences in temperature of up to 5.5 degrees C between restrained and unrestrained leaflets of the same soybean cultivar. Similarly, Marler and Lawton (1995) found that when Star Fruit (*Averrhoa carambola)* tree leaves were restrained from heliotropic movement, the effect was an increase in leaf temperature and a decrease in photochemical efficiency when compared to leaves allowed to move naturally. Differences of in leaf temperatures due to leaflet orientation can vary depending on leaf angle and color. Differences of up to 5.4 degrees C have been recorded in research by Medina et al. (1978) which is similar to other research findings.

Leaflet orientation and yield

Chang and Tagumpay (1970) found that rice plants with erect leaves were correlated with higher yields while plants with drooping leaves were correlated with lower yields. Increased yields of maize (*Zea mays*) hybrids have been associated with vertical leaf angle which allow more light penetration into the canopy (Mickelson et al., 2002; Pendelton et al., 1968; Pepper et al., 1977). Similarly leaflet orientation in soybean has been related to increased light interception and yield potential (Shaw and Weber, 1967; Wang et al., 1995). However, Isoda and Tomagae (2003) compared biomass and seed yields of a highly orienting soybean cultivar which had its upper canopy leaves restrained from flowering to harvest in contrast to the same unrestricted cultivar. The study detected no differences in biomass or seed yields between the forced “low orienting” treatment and the “high orienting” control. There were also no differences detected in photosynthetic efficiencies or photoinhibition which may indicate genotypic and/or environmental effects noted in the study as the results are contrary to previous research on the photosynthetic and photoprotective advantages of leaflet orientation (Shaw and

Weber, 1967; Prichard and Forseth, 1998; Ikeda and Mastuda, 2002; Wang et al., 1995; Jiang et al., 2006; Hirata et al., 1983; Rosa et.al., 1991; Rosa and Forseth, 1995; Kao and Tsai, 1998).

Root morphology

Development of breeding lines that have superior root systems may be an effective way to stabilize crop yields in drought-prone regions (Chaves et al., 2003; Kell, 2011). The ability of plants to resist drought has been found to be proportional to the density and extent of root development (Quizenberry, 1982). More expansive root architecture also allows plants to exploit soil mineral resources which may aid in increased nutrition, drought tolerance and yield (Lynch, 1995). A deeper and more expansive root system may allow soybean plants to efficiently access more soil area and thus more soil moisture (Pathan et al., 2007; Taylor, 1980). This would increase the ability of soybean plants to uptake water in drought stressed environments.

Hammer et al. (2009) found that root system architecture had a direct positive effect on maize biomass accumulation and yield in simulated models and field experiments. Palta et al. (2011) stated that large root systems in wheat (*Triticum aestivum*) can contribute to drought adaptation but may also result in depleting soil moisture reserves under certain conditions. Lopes and Reynolds (2010) found that increased root mass at depth of wheat isomorphic sister lines was associated with increased soil water extraction, cooler canopy temperatures and increased yield under drought conditions.

Significant variation for root size and morphology has been found in soybean (Quizenberry, 1982; Howard, 1980). Boyer et al. (1980) found that recently developed, higher yielding soybean lines had lower mid-day water deficits and larger root densities than older, lower yielding cultivars. Garay and Wilhelm (1983) found that isolines of the soybean cultivar

Harosoy which had greater root density, explored deeper into the soil profile and extracted more water during drought stress than the normal isoline. Jin et al. (2010) reported that a group of higher yielding soybean lines tended to have greater biomass, root mass and rooting depth than a group of lower yielding lines.

The plant introduction line PI 416937 has been the focus of several researchers over the past 20 years. Goldman et al. (1989) found that PI 416937 maintained a substantially higher water content, water potential, and transpiration than the cultivar Forrest when subjected to drought and aluminum stress. Sloane et al. (1990) reported that PI 416937 had superior ability to maintain transpiration, leaf turgor, and relative yield under drought stress than the popular cultivar Forrest and therefore might be an important source of drought tolerance for breeding programs. The study also suggested the possibility that PI 416937 might be able to extract more water at greater depth than the cultivar Forrest. Hudak and Patterson (1995) found that PI 416937 roots had greater mass, volume and surface area than that of Forrest. It was also noted that PI 416937 tends to have a fewer number of relatively large leaves resulting in an overall increase in leaf surface area than Forrest. PI 416937 was characterized in this study as possessing a fibrous-like root morphology with a proliferation of branches and fine rooting structures. This differs from the normally observed root morphology of soybean plants such as Forrest which are characterized as possessing a prominent tap root with few braches. It was surmised that a fibrous-like root system should be more efficient at water absorption and extraction. In a continued comparative study of PI 416937 and Forrest, Hudak and Patterson (1996) found that in addition to its larger root mass PI 416937 also had a larger lateral root spread which allowed it to exploit more soil area. It was also discovered that the rate of soil desiccation by PI 416937 was lower than that of Forrest. It was postulated that by extracting moisture at a slower rate from a larger volume of soil, soil moisture might be available to the plant over a longer period of time. Similarly, King et al. (2009) found that PI 416937 depleted soil moisture at a lesser rate than other less drought tolerant lines. Row spacing experiments indicated that increased lateral rooting ability was probably not responsible for differences in drought tolerance as evidenced by wilting responses. It was noted that additional mechanisms in addition to root morphology may be involved in the lines ability to tolerate drought conditions. This was also indicated by Fletcher et al. (2007) who found that the slow wilting plant introduction PI 416937 had the ability to limit its transpiration rates under conditions of low humidity whereas commercial soybean lines continued to increase transpiration rates under increasing vapor pressure deficits. This ability contributes to the explanation of the reduced soil moisture depletion observed in PI 416937.

A technique for evaluation and selection of the fibrous-like rooting trait was employed successfully and described in Pantalone et al. (1996a). Researchers used a peanut inverter and a visual scoring system in order to expose the root systems and phenotypically rate soybean roots. The fibrous-like prolific root system was found to have an increased capacity for symbiotic nitrogen fixing nodules. This may enhance the plants ability to maintain biological nitrogen fixation longer under periods of drought stress resulting in higher relative yields (Kohli et al., 2012). Root score was found to be positively correlated with root surface area, nodule number and nodule weight. The phenotypic evaluation method was deemed an effective and efficient process for selection of soybeans with prolific rooting systems. Mian et al. (1993, 1994) found that root mass in field grown wheat could be accurately selected by growing and rating plants in hydroponic culture. Similar unpublished results of soybean root evaluations have been conveyed in conversations but at present the methodology and efficacy of this technique is unclear.

Pantalone et al. (1996b) employed the phenotypic rating method and obtained heritability estimates and correlations related to the fibrous-like prolific rooting trait among population lines from a cross between ‘Lee 74’ × PI 416937. The frequency distribution of rooting scores approximated a normal distribution suggesting the trait is polygenic. Heritability on an entry-mean basis was found to be 0.39 while realized heritability was estimated at 0.24. These were recognized as relatively low heritability estimates but not unexpectedly so for a quantitative trait and similar to that of seed yield. The observed genetic gain in root score indicated the trait can be successfully utilized in breeding programs to improve germplasm. A positive correlation was found between root score and seed protein, indicating potential importance of the prolific rooting trait in seed protein accumulation. Although no significant correlation was found between root score and yield, seed oil or seed weight, the correlation between seed yield and rooting score was negative ($r = -0.56$) in this experiment.

Patterson and Hudak (1996) found that PI 416937 accumulated more dry matter and nitrogen than Forrest under water stress. This was attributed to the ability of PI 416937 to maintain higher leaf water potential, higher photosynthetic rates, and greater nodulation. While seed yield of Forrest was greater than PI 416937, the yield reduction under drought was less for PI 416937. This indicated that germplasm which can maintain turgor, leaf and nodule function during drought stress may reduce overall yield losses.

Pantalone et al. (1999) conducted soybean grafting experiments which included the soybean line PI 416937 in order to evaluate the effects of the shoot and rootstock on various traits. It was determined that the prolific rooting morphology of PI 416937 was regulated by the root system itself and not conditioned by the shoot. This was evidenced by the ability of the PI rootstock to maintain its prolific rooting morphology when grafted to a scion from a cultivar which did not normally possess a prolific root. Additionally rootstocks of normally tap rooted cultivars maintained that phenotype when grafted to the PI scion. Although non-significant, increases in seed protein and biomass were detected as a result of grafting the PI prolific rootstock to scions from other cultivars.

In order to investigate the potential drought tolerance contributions of shoot and root physiologies of PI 416937, experiments were conducted where rooting volumes were restricted. Results indicated that rooting characteristics such as increased root volume may allow for increased exploitation of soil moisture and the subsequent “slow wilting” phenotype of the line (Chipman et al., 2001).

In a comparative study with the cultivar Essex, Busscher et al. (2000) reported that PI 416937 possessed a greater capability to continue to produce root growth through compacted and acidic soil layers. The ability to explore into areas of harder soils may contribute to the cultivar’s slower wilting phenotype in times of drought stress.

Purcell (2006) reported that slow wilting genotypes such as PI 416937 may exhibit this phenotype due to their lower transpiration rates during periods of plentiful soil moisture. This results in more available soil moisture during dryer periods and thus slower wilting. This lower transpiration rate may contribute to lower growth rate and seed yield observed in genotypes exhibiting this trait (Ries et al., 2012).

Water Use Efficiency

Water use efficiency (WUE) of crop plants can be improved by selection for improved transpiration efficiency and harvest index. In his review of water use efficiency in crop plants, Turner (1993) noted that increasing WUE by lowering transpiration alone can lead to lower yields due in part to increased temperatures and reduced photosynthetic efficiencies. Crop production is strongly associated with total transpiration, however it may be possible to improve WUE if intrinsic photosynthetic capacity in plants can be selectively increased in plants (Van Den Boogaard et al., 1997; Udayakumar et al., 1998). Costa and Ariyawansha (1996) found that WUE rankings differed in common bean under stress as compared non stressed environments. Water use efficiency was defined in this study as the ratio of kilograms of biomass per gram of water transpired. They also found positive correlations existed between WUE, seed yield and harvest index but cautioned that other studies had reported negative correlations between WUE and overall crop productivity. Generally under water stress, less water is available for transpiration which increases WUE statistics but usually reduces overall productivity.

Purcell (2006) stated the main tenets of crop physiology are that crop mass and yield are proportional to the cumulative amount of light intercepted and the amount of water transpired by the crop during a season. Research indicates this to be true although the relationships may be more curvilinear than previously perceived. Edwards et al. (2005) found that although yield continued to increased with cumulative intercepted photosynthetically active radiation through 1100 MJ m^{-2}, 90% of maximum soybean yield can be obtained by intercepting 605 MJ m^{-2}. Similarly Purcell et al. (2007) found that while soybean yield continued to increase with cumulative transpiration through 750 mm of soil profile water, 90% of the maximum yield could be obtained by transpiring 444 mm. This is encouraging for researchers who wish to improve soybean water use efficiencies as it indicates genotypes may exist or can be developed that regulate water use and light interception in such a manner as to maximize yield while using only as much water as needed. Identification of these types of plants and their associated traits would be of great interest to researchers and plant breeders.

Transpiration estimates using heat balance sap flow measurement

Measurement of transpiration using the thermal heat balance method has been an accepted and commercially available method for more than 20 years. The advantage of this system is that it can be used to measure transpiration rates in real time under field conditions on crop plants seeded by conventional methods similar or identical to those used by producers. Early research on the efficacy and accuracy of this method varied somewhat. Cohen et al. (1993) found that under periods of high flow rates above 100 g h^{-1}, the heat balance method tended to underestimate soybean transpiration by as much as 20% in some instances. Conversely they found that corn transpiration was overestimated by the heat balance sap flow method by 25% under similar conditions. Gerdes et al. (1994) compared sap flow measurements to transpiration estimates obtained from the SOYGRO model (Jones et al., 1989) and reported that sap flow measurements overestimated transpiration rates in soybeans. Kjelgaard et al. (1997) evaluated heat balance methods for estimating transpiration of sunflower, maize, and potato. They reported high correlations overall between observed transpiration and sap flow. It was noted that in some instances of high flow rates above 100 g h^{-1} that estimates of sap flow varied from observed transpiration similar to that reported by Cohen et al. (1993). It is perhaps because of these observations that the commercially available Dynamax Flow32 Sap Flow Monitoring System Manual states that "Only over long time periods, such as 24 hours or 12 daytime hours, will transpiration and sap flow be substantially equal." Additionally it states that "The size, or duration of this difference depends on the plant species, size, and environmental conditions"(van Banvel, 2000). Overall, from prior research it appears that when herbaceous plant species such as soybeans are evaluated for transpiration rates using the heat balance sap flow method, the estimates seem to be considered generally reliable and accurate under most conditions.

Estimates of total transpiration over a longer time period are generally more accurate than rate estimates at any one point in time, especially if flow rates exceed 100 g h^{-1} .

Single plant evaluations

Physiological measurements are often performed on one or few numbers of plants due to the time consuming and complex nature of the measurement procedures. Yields from these small numbers of plants are sometimes used to infer relationships between physiological traits and seed yield. The efficacy of evaluations of yield and other traits on a single plant basis has been mostly negative (Wehner and Miller, 1984; Lunlsdorf and McVetty, 1986; Pasini and Bos, 1990) with only an occasional study showing positive correlations (Nass, 1973). The question arises as to how many single plant measurements are needed in order to have an acceptable representation of a genotype or ideotype being evaluated. Larger plots consisting of plants at densities corresponding to established recommended seeding rates are generally considered more representative and accurate than smaller plots. This is due to experimental aspects such as field variation and interplant competition effects (Fehr, 1987). However some researchers have found good correlation between small hill plots containing few plants and larger row plots (Torrie, 1962; Frey, 1965; Garland and Fehr, 1981). Fasoulas (1981) indicated that 100 single plant evaluations may be needed to obtain satisfactory results. This may vary depending on the crop species, plant spacing, and trait being measured.

Development and use of near isogenic lines

Near isogenic lines are useful genetic stocks for evaluating effects of genetically controlled traits. The goal of isogenic line development is to create plants that are genetically and phenotypically similar in all respects except for the trait(s) of interest. Comparisons for effects can then be made between near isogenic genotypes which possess differing phenotypic attributes. Near isogenic lines are commonly produced by recurrent back crossing (Fehr, 1987). This method is best suited to genotypic traits controlled by one or few genes. Near isogenic lines can also be produced by descent. This involves successive generations of inbreeding, usually through modified single seed descent methodology (Brim, 1966), in order to ensure some level of homozygosity and genetic similarity within each developed line. This is followed by detection and separation of those advanced homozygous lines which are still exhibiting segregation for the trait of interest (Haley et al., 1994; Yang et al., 1995; Mickelbart et al., 2003; Glover et al., 2004, Yamanaka et al, 2006). The development of near isogenic lines by descent can be particularly useful when dealing with a trait which is quantitatively controlled.

Use of grafts in soybean research

Grafting procedures which combine scion and rootstocks of differing phenotypes can be useful for evaluating the effects of those phenotypes both singly and in combination. Grafts have been used in plant research among many plant species including soybean (Hamaguchi et al., 1993; Pantalone et al., 1999; Bachman and Nickel, 1999; Przepiorkowski and St. Martin, 2003; Voung and Hartman, 2003) in order to evaluate the effects of the upper and lower portions of plant genotypes on a variety of characteristics. The creation of grafted plant combinations can be particularly useful when dealing with traits which are quantitatively controlled and therefore may be difficult to obtain due to time and resource limitations.

Goals of research

The concept of ideotype breeding is basically identifying morphological traits that affect overall fitness, desirability and yield in a positive manner and then using those traits to assist in selection of superior performing genotypes (Donald, 1968). Ideotypes will vary depending on the species, environment, and overall goals of the breeding project. Many early studies involving this concept have involved canopy and root characteristics. Progress under this concept has been slow as many of the traits are complex and controlled by many genes. Their effects on yield are small and therefore it is often difficult to prove a causal relationship; additionally they may be linked to undesirable traits (Hamblin, 1993).

The goal of this research is to investigate the effects of leaflet orientation and prolific rooting, both singly and in combination, on water use, yield, leaf temperature, photosynthesis biomass production and other agronomic traits in soybean. Preliminary screening of available germplasm was completed and crosses made between parent lines, which resulted in populations segregating for the two traits. As these populations were advanced, near isogenic lines were identified and developed which were similar in all respects except the two traits of interest. Analyses involving plant grafting, population traits, near isogenic line sets, and restricted leaf canopy procedures were used as the basis for contrasting the effects of the two traits. Plant material was evaluated for whole plant transpiration rate, leaf area, yield, leaflet temperature, leaflet orientation, root morphology, photosynthetic rate, and other agronomic traits.

This research may provide information which will allow increases in productivity and sustainability of soybeans in response to drought which impacts the crop on a global scale. It may also provide basic knowledge, understanding, and characterization of two key physiological mechanisms and adaptation responses to drought stress in soybeans: leaflet orientation and root morphology. This may allow breeding strategies to be developed and employed which will decrease the impact of drought stress and water availability. The understanding and application of these mechanisms could enhance economic opportunities for agricultural producers through increased and stabilized yields in water scarce environments. This in turn could enhance the supply of food and reduce the impact on the environment by decreased water consumption by the crop or by enhanced water use efficiencies.

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Part I

Leaflet Orientation and Root Morphology Trait Combination Effects on Transpiration, Seed Yield, and other Agronomic Traits in Soybean via Reciprocal Grafts

Abstract

Drought is considered the single most important abiotic stress that adversely affects soybean yield. Adaptive traits which condition dehydration avoidance include those which minimize excessive temperature stress and water loss and those which maximize water uptake. Two potential traits of interest are leaflet orientation and root morphology. Leaflet orientation has been shown to reduce leaflet temperatures and water loss while root morphology has been related to slower wilting phenotypes. The objective of this study was to investigate the effects of leaflet orientation and rooting morphology on whole plant transpiration, yield, and water use efficiency in soybean. The experiment was conducted at Knoxville, TN (35.89 lat., 83.96 long.) during the 2003 growing season. Three soybean cultivars were chosen: USG 5601T, PI 416937 and Williams 82 which differed in leaflet orientation, root morphology, and other characteristics. Twelve treatments consisting of non-grafted plants of each cultivar, self grafts and reciprocal grafts of scion and rootstocks were made among the three cultivars. Whole plant transpiration of plants was measured on several successive days via a Dynamax Flow 32 Sap Flow Monitor™ when the plants were in the R4-R6 stage of growth. Data for leaflet orientation, rooting morphology, seed yield, photosynthetically active radiation (PAR), solar radiation (SAR), plant height, seed size, and seed protein and oil concentration were also recorded for all treatments. No significant differences were detected between the non-grafted and self grafted treatments of each line for leaflet orientation, root morphology, whole plant transpiration, water use efficiency, seed size, or plant height. Significant differences detected for seed yield and seed protein and oil concentration between the self grafted and non-grafted treatments of PI 416937 may indicate an effect due to grafting technique, which in this study, appeared to be limited to these three traits of this one line. Leaflet orientation, seed yield, water use efficiency, seed size, plant height, and

seed protein and oil concentration were all significantly affected by the scion treatment in a manner reflective of the scion donor line, indicating that these traits were conditioned predominately by the shoot portion of the plant. Root morphology scores were not significantly different ($p \leq 0.05$) among scion treatments, indicating that the root morphology is conditioned independently from the upper part of the plant. Whole plant transpiration was not significantly different ($p \leq 0.05$) among scion treatments. Root morphology score was the only trait which was significantly different ($p \leq 0.05$) among the rootstock treatments. None of the other measured traits were significantly different when comparing among rootstock treatments averaged across scion treatments indicating these traits were unaffected by the rootstock treatments. Combinations of high or low leaflet orientation with normal or prolific rooting morphology had no significant ($p \leq 0.05$) discernable effect on whole plant transpiration. The high leaflet orienting line scion treatment, USG 5601T, had higher yield and used less water per unit yield than the low leaflet orienting line scion treatment, PI 416937. Although this is anecdotal due to the genetic differences of the lines, it may lend some support to the idea that plants with high leaflet orientation are adapted to have increased yields with better water use efficiencies. The lack of effect of the PI 416937 prolific rootstock on whole plant transpiration across scion treatments supports findings that the differential slow wilting and transpirational attributes of this line may not be as related to the root morphology as previously speculated. Since there was abundant soil moisture at the experimental location during the 2003 growing season, it is not known whether the leaflet orientation or prolific rooting traits would have been beneficial during a moisture stressed environment.

CHAPTER I

Introduction

Research into plant responses to water stress is becoming increasingly important, as most climate-change scenarios suggest an increase in arid land area in many regions of the world. On a global basis, drought in conjunction with high temperatures and solar radiation constitutes the most important environmental factors limiting crop productivity. Agriculture is a major consumer of water resources in many regions of the world. As human population increases water will become a scarcer commodity. A better understanding of drought tolerance in plants is vital for improved management practices and breeding strategies (Chaves et al., 2003).

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many growing regions of the world. Drought is considered the single most important abiotic stress that adversely affects soybean yield by approximately 40% (Pathan et al., 2007). Consequently, drought tolerance is a highly sought after trait in soybean cultivars. Drought tolerance is a complex response and is conditioned by the interaction of several genetic traits of the plant to environmental conditions (Chaves et al., 2003). Knowledge of these trait processes is needed not only for understanding plant resistance to drought stress but also for improved crop management and breeding techniques.

Many of the traits that are attributed to plant adaptation during drought such as phenology, root size and depth, and hydraulic conductivity are associated with plant development and structure and are constitutive rather than stress induced. A considerable part of plant resistance to drought is the ability to dissipate or avoid excess radiation. The nature of the mechanisms responsible for leaf photoprotection, especially those related to thermal dissipation and oxidative stress are therefore of great interest. A desirable plant type would be one that could endure drought conditions while maintaining a higher level of productivity by avoiding tissue dehydration, maintaining tissue water potential and photosynthesis as high as possible. Adaptive traits which condition dehydration avoidance include those which minimize excessive water loss and maximize water uptake. Water loss can be reduced by reducing light absorbance via steep leaf angles. Water uptake can be maximized by increasing the rooting volume and/or depth (Chaves et al., 2003). Two potential traits of interest are therefore leaflet orientation and root morphology. Leaflet orientation addresses the need to reduce water loss and root morphology addresses the ability to maximize water uptake.

Leaflet orientation

Many species of plants are capable of leaf movements in response to external stimuli (Ehleringer and Forseth, 1980). Leaf movement in response to light, known as heliotropism, can be classified as either diaheliotropic (light seeking) or paraheliotropic (light avoiding). Plants exhibiting diaheliotropism orient the plane of the leaf blade perpendicular to incident light rays, while plants exhibiting paraheliotropism orient the plane of the leaf blade parallel to incident light rays. Soybean exhibits both diaheliotropic and paraheliotropic movements, with the degree of movement being dependent on genotypic response (Wofford and Allen, 1982) and various levels of environmental stimuli (Ehleringer and Forseth, 1989; Rosa and Forseth, 1995).

Paraheliotropism and diaheliotropism provide a means by which the plant can alter the arrangement of its leaves in order to gain maximum benefit from the environment. Advantages of changing leaf angle and light absorbance include increased total canopy light interception (Kawashima, 1969 a,b; Wang et al., 1994), increased photosynthetic efficiency (Prichard and

Forseth, 1988 He et al., 1996), and, increased yield (Wang et al., 1995; Pendleton et al., 1968). Leaflet orientation can also reduce leaf temperature (Forseth and Teramura, 1986; Isoda and Wang, 2002) which can reduce excessive transpiration rates (Bielenberg et al., 2003; Berg and Heuchelin, 1990). Additionally paraheliotropism can reduce photoinhibition (Hirata et al., 1983; Jiang et al., 2006), and increase water use efficiencies (Rosa et al., 1991; Kao and Tsai, 1998). In soybean, this phenomenon may be a mechanism to reduce water loss while maintaining some level of productivity as reported by Meyer and Walker (1981). Paraheliotropic leaf movements reduce transpirational water loss by lowering light interception of leaves, consequently improving water status and lowering leaf temperature.

Research conducted at the University of Tennessee demonstrated that soybean cultivars differ in their ability to orient leaflets during the course of the day (Wofford and Allen, 1982). Most cultivars exhibit high leaflet orientation (paraheliotropism) and move their leaves during the course of the day such that the leaves have maximum exposure to the sun in the early and late parts of the day, but during mid-day the leaves are oriented parallel to sunlight such that the surface of the leaves has minimum exposure to the sun. A lesser number of cultivars exhibit low leaflet orientation whereas the leaf surface remains relatively flat and changes little relative to the position and intensity of sunlight, even during the mid-day period of highest irradiance. These "low leaflet orienting" types are therefore relatively less paraheliotropic.. In a study of the cultivar Essex (high leaflet orientation) and Dare (low leaflet orientation), the two cultivars produced about equal yields; however Essex used about one-half the amount of water as Dare during the growing season (Paris, 1997).

In work with soybean, Lugg and Sinclair (1981) found that upper leaflets of the canopy maintained a higher net photosynthetic rate per unit leaf area than did the lower leaflets. This seemed to be mostly due to shading, as the lower leaves were found to have photosynthetic rates similar to upper canopy leaves when unshaded. Kawashima (1969 a,b) found that soybean leaflets exhibiting paraheliotropism in the upper canopy allowed light to penetrate more deeply into the canopy, increasing photosynthetic output of the lower leaves, thus allowing total photosynthetic efficiency of the plant to be improved. Vertical leaf angles decrease the amount of solar radiation intercepted by the leaf. However photosynthetic rate response in plants to solar radiation is nonlinear and saturates below the intensity of direct ambient sunlight (van Zanten et al., 2010). Soybean plants are reported to maximize their photosynthetic rates at less than one-third the amount of full sunlight according to Beuerlein and Pendleton (1971). Vertical leaflet orientation increases overall photosynthesis by allowing the upper canopy leaves to continue to photosynthesize under lower than ambient sunlight while also allowing lower canopy leaves to contribute at an increased rate (van Zanten et al., 2010).

Kao and Tsai (1998) studied leaf movements in three soybean species (*Glycine soja, G. tomentella, and G. tabacina)* and found that paraheliotropism seemed to enhance water use efficiency and decrease the risk of photoinhibition in plants under water stress. Grant (1999) found that soybean plants that exhibit paraheliotropism are able to reduce UV-B irradiance in contrast to plants that do not orient leaflets. Ikeda and Matsuda (2002) studied photosynthetic efficiency differences in soybean leaves which were restrained from orienting versus naturally orienting. Their results indicated that paraheliotropic leaflet movements are an adaptation which optimizes net leaflet photosynthesis.

Isoda et al. (1992, 1993) found that the paraheliotropic movements of soybean leaflets regulate light interception and reduce leaf temperature. Isoda and Wang (2002) studied leaf temperature and transpiration rates of cotton versus soybean and found that soybean plants were able to reduce leaf temperatures and transpiration rates. This was attributed to the soybean cultivars ability to orient its leaves in a paraheliotropic manner. In a study involving restrained and unrestrained soybean leaflets, Isoda and Tomagae (2003) found differences in temperature of up to 5.5 degrees C between restrained and unrestrained leaflets of the same soybean cultivar.

Chang and Tagumpay (1970) found that rice plants with erect leaves were correlated with higher yields while plants with drooping leaves were correlated with lower yields. Increased yields of maize hybrids have been associated with vertical leaf angle which allow more light penetration into the canopy (Mickelson et al., 2002; Pendleton et al., 1968; Pepper et al., 1977). Similarly leaflet orientation in soybean has been related to increased light interception and yield potential (Shaw and Weber, 1967; Wang et al., 1995). However, Isoda and Tomagae (2003) compared biomass and seed yields of a highly orienting soybean cultivar which had its upper canopy leaves restrained from flowering to harvest in contrast to the same unrestricted cultivar. The study detected no differences in biomass or seed yields between the forced “low orienting” treatment and the “high orienting” control. There were also no differences detected in photosynthetic efficiencies or photoinhibition which may have been influenced by genotypic and/or environmental effects noted in the study as the results are contrary to previous research on the photosynthetic and photoprotective advantages of leaflet orientation (Shaw and Weber, 1967; Prichard and Forseth, 1998; Ikeda and Mastuda, 2002; Wang et al., 1995; Jiang et al., 2006; Hirata et al., 1983; Rosa et al., 1991; Rosa and Forseth, 1995; Kao and Tsai, 1998).

Root Morphology

Development of breeding lines that have superior root systems may be an effective way to stabilize crop yields in drought-prone regions (Chaves et al., 2003; Kell, 2011). The ability of plants to resist drought has been found to be proportional to the density and extent of root development (Quizenberry, 1982). More expansive root architecture also allows plants to exploit soil mineral resources which may aid in increased nutrition, drought tolerance and yield (Lynch, 1995). A deeper and more expansive root system may allow soybean plants to efficiently access more soil area and thus more soil moisture (Pathan et al., 2007; Taylor, 1980). This might increase the ability of soybean plants to uptake water in drought stressed environments.

Significant variation for root size and morphology has been found in soybean (Quizenberry, 1982; Howard, 1980). Boyer et al. (1980) found that more recently developed, higher yielding soybean lines had lower mid-day water deficits and larger root densities than older, lower yielding cultivars. Garay and Wilhelm (1983) found that isolines of the soybean cultivar Harosoy which had greater root density, explored deeper into the soil profile and extracted more water during drought stress than the normal isoline. Jin et al. (2010) reported that a group of higher yielding soybean lines tended to have greater biomass, root mass and rooting depth than a group of lower yielding lines.

A soybean plant introduction cultivar from Japan, PI 416937 (Houjaku Kuwasu), which exhibits significant drought and aluminum tolerance (Goldman et al., 1989; Sloane et al., 1990; Hudak and Patterson, 1995) has been the focus of several researchers over the past 20 years. This soybean line has also been characterized as possessing an extensive fibrous-like prolific root morphology which differs from the normal tap root of most soybeans (Hudak and Patterson, 1995; Pantalone et al., 1996, 1999). Several studies have indicated the unique rooting morphology of PI 416937 as a major component of its ability to tolerate drought (Hudak and Patterson, 1995, 1996; Chipman et al., 2001). The prolific rooting morphology of the PI has been shown to support increased numbers of nitrogen fixing nodules (Pantalone et al., 1996; Patterson and Hudak, 1996) and enhanced nitrogen fixation (Marlow, 1993) which may contribute to drought tolerance. The PI root system has also been shown to penetrate and continue to grow through hard soil layers that were impenetrable to other cultivars (Busscher et al., 2000). In addition to its root morphology, studies have indicated that PI 416937 may also tolerate drought by means of its osmotic regulation which appears to be somewhat different than that of other soybean cultivars. Fletcher et al. (2007) reported that PI 416937 demonstrated the ability to limit its transpiration rate under conditions of vapor pressure deficits associated with low humidity. Other genotypes continued to increase transpiration rates under increasing vapor pressure deficits. This contributes to the explanation of decreased soil desiccation by PI 416937 plants observed by Hudak and Patterson, (1996) and King et al. (2009).

Water Use Efficiency

Water use efficiency of crop plant can be improved by selection for improved transpiration efficiency and harvest index (Turner, 1993). Purcell (2006) stated the main tenets of crop physiology are that crop mass and yield are proportional to the cumulative amount of light intercepted and to the amount of water transpired by the crop during a season. Research indicates this to be true although the relationships may be more curvilinear than previously perceived. Edwards et al. (2005) found that although yield continued to increased with cumulative intercepted photosynthetically active radiation through 1100 MJ m^{-2}, 90% of maximum soybean yield can be obtained by intercepting 605 MJ m^{-2}. Similarly, Purcell et al. (2007) found that while soybean yield continued to increase with cumulative transpiration through 750 mm of soil profile water, 90% of the maximum yield could be obtained by transpiring 444 mm. This is encouraging for researchers who wish to improve soybean water use efficiencies as it indicates genotypes may exist, or can be developed, that regulate transpiration and light interception in such a manner as to maximize yield while using only as much water as needed. Identification of these types of plants and their associated traits would be of great interest to plant breeders and other researchers.

Use of grafts in soybean research

Grafting procedures which combine scion and rootstocks of differing phenotypes can be useful for evaluating the effects of those phenotypes both singly and in combination. Grafts have been used in plant research among many plant species including soybean (Hamaguchi et al., 1993; Pantalone et al., 1999; Bachman and Nickel, 1999; Przepiorkowski and St. Martin, 2003; Voung and Hartman, 2003) in order to evaluate the effects of the upper and lower portions of plant genotypes on a variety of characteristics. The creation of grafted plant combinations can be particularly useful when dealing with traits which are quantitatively controlled and therefore may be difficult to obtain due to time and resource limitations.

The objective of this research is to investigate the effects of leaflet orientation and prolific rooting, both singly and in combination, on whole plant transpiration, yield, and water use efficiency. Effects on other agronomically important traits such seed size, plant height, seed protein and oil will also be evaluated. Knowledge of these effects may provide information which may allow increases in productivity and sustainability of soybean in response to drought which impacts the crop on a global scale. It may also provide basic knowledge, understanding, and characterization of two key physiological mechanisms and adaptation responses to drought stress in soybean, leaflet orientation and root morphology. This may allow breeding strategies to be developed and employed which will decrease the impact of drought stress and water availability. The understanding and application of these mechanisms could enhance economic opportunities for agricultural producers through increased and stabilized yields in water scarce environments. This in turn could enhance the supply of food and reduce the impact on the environment by decreased water consumption by the crop or by enhanced water use efficiencies.

CHAPTER II

Materials and Methods

An experiment was conducted at Knoxville, TN USA (35.89 lat., -83.96 long.) during the 2003 growing season using grafted plants to evaluate various combinations of leaflet orientation and root morphology traits on water use characteristics. Three soybean cultivars were chosen for this study: USG 5601T, PI 416937, and Williams 82 (Pantalone et al., 2003; Pantalone et al., 1999; Bernard and Cremeens, 1988). USG 5601T is a recently released high yielding, maturity group V, determinate cultivar that has high leaflet orientation and normal tap root morphology. PI 416937 is a maturity group VI, determinate plant introduction that has low leaflet orientation and prolific, fibrous-like root morphology. Williams 82 is an improved earlier generation, maturity group III, indeterminate cultivar that has intermediate leaflet orientation and normal root morphology.

Twelve grafting treatments were used in this experiment. Reciprocal grafts of all possible combinations of scion and rootstocks were made among the three cultivars. Self grafts and non-grafted plants of each cultivar were also included in the study in order to evaluate effects due to the grafting procedure. The grafting methodology employed in this experiment was similar in most regards to the procedures described in Pantalone et al. (1999). Soybean seeds were planted in polystyrene trays consisting of 32 cells (Model TR32A, Speedling Inc., Sun City, FL) filled with soilless growth medium (PRO-MIX BX; Premier Tech Horticulture, Quebec, Canada). Grafts were initiated 5 to 10 days after planting, when the apical meristem had reached approximately 5 cm above the surface. The hypocotyl was severed approximately 2.5 cm below the cotyledon with a scalpel. The upper excised portion of the plant served as the scion while the lower portion remaining in the growth media became the rootstock. A vertical incision of approximately 6 mm in depth was made into the top center of the rootstock. The severed end of the scion was trimmed to form a V shaped wedge which was then inserted into the rootstock incision. The graft union was secured using a small clothespin-like fastener (Model No. 38049, Amscan Inc., Elmsford, NY) which served as a grafting clip. Grafts were placed on greenhouse benches which received no direct sunlight. An irrigation system (Mist Sprayer Part 67191, Timer Model HT2, Orbit Irrigation Products, Bountiful, UT) delivered two minutes of fine mist to the grafted plants every two hours for the first four days (Fig. 1.1, all tables and figures for each part of this dissertation are located in appendices at the end of each part). Plants were watered daily as needed for the next four days and then growth media was allowed to dry slightly the next two days. The slight drying of the growth media allowed for easier intact removal of the grafted plant and its associated growth media from the polystyrene trays. Ten to 14 days after the grafting procedure, the graft unions were deemed successful and stable and the grafting clips were removed.

The self grafted, reciprocally grafted, and non-grafted seedlings were transplanted into the field on 9 June, 2003. A randomized complete block design with four replications was implemented on an Etowah Silt Loam soil (fine-loamy, siliceous, semiactive, thermic Typic Paleudult) at the East Tennessee Research and Education Center in Knoxville, TN. Twelve plants of each grafting treatment were placed into each of the four replications. Plants were spaced approximately 15 cm apart in the center of a 3m single-row plot with 76.2 cm spacing between each row. The two ends of each plot (approximately 45 cm) were seeded with soybeans approximately 3 cm apart in order to provide plant competition similar to typical field production environments and to reduce the incidence of stem girdling to the grafted plants by insects.

Whole plant transpiration rates were measured on several successive days using the Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stage of growth (R4-R6). Although this measurement may not be representative of transpiration over the growing season, it is deemed important as it represents the period in which seed yield and seed quality constituents are developed and water use is at or near its peak (Wilson, 2004; Heatherly and Elmore, 2004). Consequently, this is also the approximate period when leaflet orientation values were found to be at their highest by Wofford and Allen (1982). Dynamax model SGA9 Flow32 System Dynagauges were used to connect each plant to the system as the approximate 9mm diameter size of the Dynagauge would properly fit around the lower stem of grafted plants just above the graft union. Each plant was marked with a durable tag for identification purposes later in the season. The stem diameters were measured and cleaned. The interior of the Dynagauge sensor was lubricated with a very thin film of Dow Corning 4 Electrical Insulating Compound (Dow Corning Corp, Midland, MI) and then placed around the stem in such a manner as to ensure that the thermocouples and heater strip of the sensor were in direct contact with the stem. The top and bottom of the sensor was then sealed with Elmer's Poster Tack adhesive putty (Elmer's Products Inc., Westerville, OH). The sensor was then wrapped with a sheet of Reflectix double reflective insulation (Reflectix Inc., Markleville, IN) measuring approximately 14 cm x 33 cm which provides two layers of insulation. The insulation was held in place by placing a cable tie near the top, bottom and middle of the sensor in a manner such that the insulation was secure but with minimal pressure being applied to the stem. The system was mounted to a vertical cart with wheels for easier transportation within the field. The battery and data cables were placed in a large tool box also mounted to the cart. Additionally, a solar panel was attached to the cart to extend the battery life and operating capacity of the system (Fig. 1.2). Whole plant transpiration data were collected on two plants from each grafting treatment in each replication over a period of two to four days depending on the environmental conditions. The goal was to collect data from a 24 hour period when the conditions were mostly sunny; therefore some measurements covered a longer period of time due to cloudy days after the system was installed on the plant material. Transpiration data (grams of water per 24 hour period) from a single, mostly sunny day from each replication of the experiment were used in this analysis. Data collected on other days were not utilized due to factors such as sensor malfunctions and/or environmental conditions. Whole plant transpiration data were collected on replications one through four on 15 August, 27 August, 31 August and 9 September, 2003 respectively.

Photosynthetically active radiation (PAR), solar sadiation (SAR), and soil moisture were recorded at the field location using a Hobo® weather station equipped with H21-001 data logger, S-LIA-M003 PAR, S-LIB-M003 pyranometer, and S-SMA-M003 soil moisture sensors (Onset Computer Corporation, Pocasset, MA).

Leaflet orientation score for each plot was taken on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining and angle parallel to the horizontal plane (Fig. 1.3). Leaflet orientation scores were taken between the hours of 1300 and 1500 each day during the measurement of whole plant transpiration for each replication as this is the period of the day in which the differential leaflet orientation was at its highest (Wofford and Allen, 1982).

Root morphology scores were obtained by removing the root system of intact plants from the soil and visually rating each set of plants for the phenotype in a similar manner described by Pantalone et al. (1996). Root morphology score was rated on a scale of 1 to 5 with 1 being the condition of the plant possessing a normal tap root with few lateral roots and 5 being the condition of the plant possessing a prolific root mass with many fibrous-like lateral branching roots (Fig. 1.4). Concurrent with the whole plant transpiration measurements for each treatment replication, three plants from each plot were carefully excised from the soil environment using a hand shovel. The soil was then removed by submerging the roots in water with moderately gentle hand agitation. Once the majority of the soil was removed, the root systems were given a score as a group in each treatment replication.

The plants which were tagged and measured for whole plant transpiration were harvested and threshed at maturity using an Almaco BT-14 belt thresher (Almaco, Nevada, IA). The amount of water transpired by the treatment in a 24 hour period during seed fill was divided by the grams of seed produced by that plant in order to obtain an estimate of water use efficiency. The seed size was measured by obtaining the weight of 100 seed. Approximately 40 grams of seed from each treatment replication was ground into a fine, uniform flour using a Knifetec 1095 Sample Mill set for a total of 20 seconds on each sample. Protein and oil analyses of the soy flour were performed on a Foss Model 6500M NIR analyzer (Foss NIRSystems Inc., Laurel, MD). Average plant heights were also recorded in all four replications. All analyses were performed using SAS Proc Mixed with the grafting treatments considered as fixed effects and the replications as random effects (SAS User Guide 9.1.3, 2006). Least squares means with mean separation and average LSD values were obtained using the SAS macro written by Saxton (1998).

CHAPTER III

Results and Discussion

More than 95% of the attempted grafts were successful using the modified grafting procedure of Pantalone et al. (1999). The use of the relatively inexpensive grafting clips increased the speed and efficiency of the grafting procedure without the need of additional support structures or comparatively cumbersome use of paraffin wax film to facilitate the grafting union. The self grafted and non-grafted plants were included in order to evaluate effects due to the grafting procedure itself (Pantalone et al., 1999, White and Castillo, 1989).

Whole plant transpiration curves followed a similar pattern on most days with measurable transpiration beginning at approximately 0800h, peaking at 1500h and ceasing at 2000h. There were differences in the overall shape of the transpiration curves on different days (Fig 1.5). These variations are due to environmental conditions such as passing cloud cover which causes a reduction in sunlight and therefore PAR and SAR. The shape and magnitude of the transpiration curves were similar and highly related to the PAR curves on any given day. This indicates the dominant role of sunlight in soybean transpiration and is similar to results reported by Gerdes et al. (1994). Cloud cover reduces amount of irradiance to the leaf, reducing air and leaflet temperatures and therefore transpiration. SAR curves were also similar to overall transpiration curves but tended to have larger variations than PAR curves (Fig. 1.6). This is likely due to the fact that SAR measurements record total solar radiation in contrast to PAR measurements which record only the spectrum of radiation known to be involved in photosynthesis.

No significant differences were detected between the non-grafted and self grafted treatments of each line for leaflet orientation, root morphology, whole plant transpiration, water use efficiency, seed size, or plant height (Figs. 1.7, 1.8). Therefore, it appears that in regards to those traits, the grafting procedure itself had no significant effect. However, there were significant differences detected for seed yield (25.7 v. 36.5), seed protein (42.8 v. 44.5%) and oil (15.8 v. 14.6%) between the self grafted and non-grafted treatments of PI 416937. This is contrary to earlier findings by Pantalone et al. (1999) and may indicate an effect due to grafting technique, which in this study, appeared to be limited to these three traits of this one line (Fig. 1.8).

Significant differences were detected among the 12 treatments for all measured traits (Figs. 1.7, 1.8). It is of interest to note that there were significant differences between the measured trait means of the individual lines used in this study. This is evidenced by the non-grafted treatment trait means of leaflet orientation (1.4, 2.5, 4.8), root morphology (1.1, 1.4, 4.8), whole plant transpiration (548.1, 313.3, 544.8), seed yield (50.7, 36.4, 36.5), seed size (15.4, 15.1, 17.8) and plant height (70.5, 91.4, 61.0) for USG 5601T, Williams 82, and PI 416937, respectively. If no differences existed among the lines it would be virtually impossible to ascertain differences due to a scion or rootstock portion of those plants. Some of these differences were known and expected, such as the higher yield of USG 5601T, the larger seed size of PI 416937, and the taller plant height of Williams 82.

In order to evaluate the effect of the scion treatment on the measured traits, the data from the non-grafted plants were dropped and the remaining data analyzed across different rootstocks. Leaflet orientation, seed yield, water use efficiency, seed size, plant height, seed protein and oil were all significantly effected by the scion treatment (Figs. 1.9, 1.10). These traits tended to have the same relative phenotype as that of the scion treatment regardless of the rootstock treatment. For example when USG 5601T scions were grafted onto Williams 82 or PI 416937 rootstocks, the leaflet orientation, seed yield, water use efficiency, seed size, plant height, seed protein and oil were not significantly different from the USG 5601T self grafted plants (Figs. 1.7, 1.8). This indicates that in regard to these traits, it is the plant shoot that predominately determines the differences in phenotype. Root morphology scores were not significantly different ($p \leq 0.05$) between scion treatments averaged across rootstocks (Fig. 1.9). This is due to the lack of effect that scion treatments had on rootstock morphology. For example, when USG 5601T and Williams 82 scions were grafted onto the PI 416937 rootstocks, the roots continued to exhibit prolific rooting scores equal to that of the PI 416937 self grafted plants (Fig. 1.7). This indicates that the root morphology is conditioned independently from the plant shoot which is in agreement with results published by Pantalone (1999). Whole plant transpiration was not significantly different ($p \leq 0.05$) among scion treatments (Fig. 1.9). This indicates that all three scion cultivar treatments transpired roughly the same amount of water regardless of the leaflet orientation differences. This further indicates that the difference in water use efficiency of the PI 416937 scion treatment is most likely due to the lower yield of this less improved genotype and not necessarily to the attributes of its leaflet orientation. It is also of interest to note that the Williams 82 non-grafted line had significantly lower whole plant transpiration than the other two lines in this study (Fig 1.8). When the Williams 82 scion treatment was averaged across rootstocks, the whole plant transpiration was lower but failed to meet significance as the Pr>F value was 0.15 (Fig. 1.9). It is also important to note that Williams 82 is earlier maturing than the other two lines and this may have had an impact on the relative whole plant transpiration values.

In order to evaluate the effect of the rootstock treatment on the measured traits, the data from the non-grafted plants were again dropped and the remaining data analyzed across different scions. This analysis enabled evaluation of the effect of each rootstock treatment when averaged across all three scions. Root morphology score was the only trait which was significantly different ($p \leq 0.05$) among the rootstock treatments (Fig. 1.11). This indicates again that root morphology is conditioned by the rootstock independently of the scion treatments. None of the other measured traits were significantly different when comparing among rootstock treatments averaged across scion treatments (Figs. 1.11, 1.12). This indicates that leaflet orientation, whole plant transpiration, seed yield, water use efficiency, seed size, plant height, and seed protein and oil concentration were unaffected by the rootstock treatment regardless of the differences in root morphology. Since the growing season in 2003 had abundant moisture, it is not known whether the leaflet orientation or prolific root morphology traits would have been more differentiating in a moisture stressed environment.

CHAPTER IV

Conclusions

The goal of this study was to physically construct via grafting, combinations of leaflet orientation and root morphology in order to determine if these trait combinations contributed to differences in transpiration, yield, water use efficiency and other agronomic traits.

No discernable effects or patterns were detected in this study to indicate a change in water use by these two traits singly or in combination. Combinations of high or low leaflet orientation with normal or prolific rooting morphology had no significant ($p \leq 0.05$) effect on whole plant transpiration (Fig. 1.7). Although rootstock treatment seemed to have no effect on any of the measured traits other than the root morphology itself, the scion treatments had significant effects on leaflet orientation, seed yield, seed size, plant height, and seed protein and oil concentration. Since the three lines used in this study are all genetically different and arise from different generations of soybean improvement programs, these differences are likely due to the genetic differences overall and cannot, with certainty, be attributed to any differing morphological attribute such as leaflet orientation or rooting morphology.

The high leaflet orienting line scion treatment, USG 5601T, had higher yield and used less water per unit yield than the low leaflet orienting line scion treatment, PI 416937 (Fig. 1.8). However, USG 5601T also seemed to transpire at a higher rate (Fig 1.9). This observation is in agreement with research findings which have shown yield to be proportional to the cumulative amount of water transpired and light intercepted during a season (Purcell 2006). Although this observation is somewhat anecdotal due to the genetic differences of the lines, it may lend some support to the idea that plants with high leaflet orientation are adapted to have increased yields

(Wang et al., 1995; Pendleton et al., 1968) with better water use efficiencies (Rosa et al., 1991; Kao and Tsai, 1998).

The lack of effect of the PI 416937 prolific rootstock on whole plant transpiration across scion treatments supports findings that the differential slow wilting and transpirational attributes of this line (Purcell, 2006; Fletcher et al., 2007; King et al., 2009) may not be as related to the root morphology as previously speculated (Hudak and Patterson, 1995, 1996; Chipman et al., 2001).

Further research investigating the effects of the leaflet orientation and rooting morphology traits is being conducted on population lines and near isogenic lines developed from the cross of USG 5601T × PI 416937.

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Trade names or commercial products were mentioned solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the University of Tennessee.

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APPENDIX A

Part I

Figures

Figure 1.1. Grafting technique: a) cutting hypocotyl, b) centered vertical incision to rootstock, c) scion end trimmed to a V shaped wedge (also visible in b), d) insertion of scion into rootstock, e) graft union secured by "grafting clip", f) misting system to keep treatments moist.

Figure 1.2. Use of Dynamax Flow32 System (fitting Dynagauges to soybean stem): a) each plant marked with durable tag for later identification, b) stem diameter measured and recorded, c) stem cleaned of dirt and debris, d) Dynagauge sensor placed around stem with top and bottom sealed with adhesive putty to prevent water and insect infiltration, e) insulating bubble wrap foil placed around Dynagauge (3 layers) and held in place with cable ties securely but with only light pressure, f) part of the Dynamax Flow 32 Sap Monitoring System setup as used in the field experiment showing the attachment to an upright cart for greater mobility, deep cycle marine battery and data link cable inside tool box at bottom, and portable computer for uploading program parameters and collecting data.

USG 5601T PI 416937 1.5 score 4.5 score

Figure 1.3. Differences in leaflet orientation at different times of day. Leaflet orientation score is a phenotypic rating on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining an angle parallel to the horizontal plane.

USG 5601T PI 416937

Figure 1.4. Visual rating scale used in scoring root morphology. Root morphology score is a phenotypic rating on a scale of 1 to 5 with 1 being the condition of the plant possessing a normal tap root with few lateral roots and 5 being the condition of the plant possessing a prolific root mass with many fibrous-like lateral branching roots.

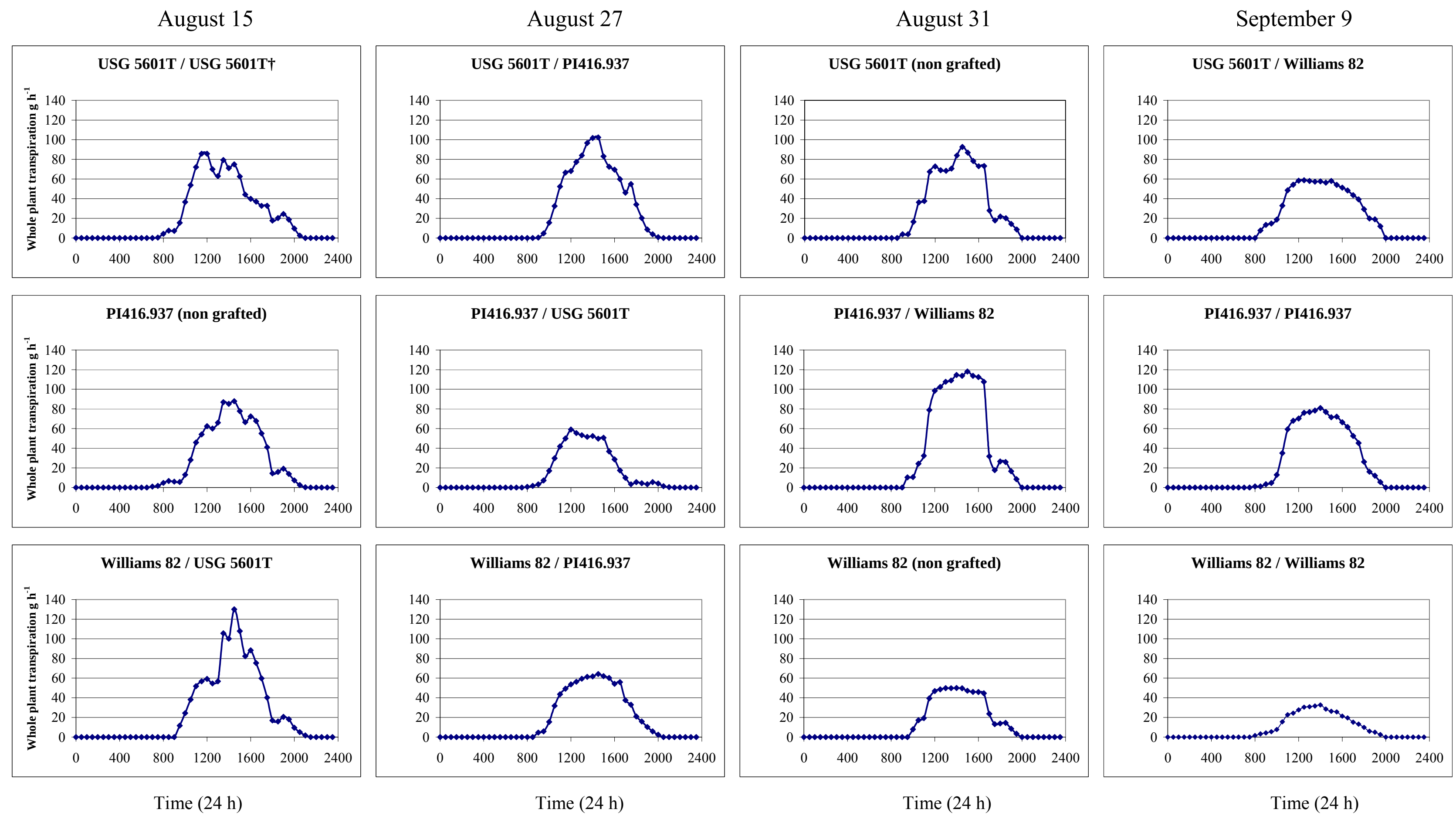

Figure 1.5. Whole plant transpiration measurements of reciprocal grafts, self grafts and non-grafted treatments among three soybean cultivars recorded during four different 24 hour periods. Although treatment graphs displayed are from different days, each is representative of the total average flow over the entire experiment. Each of the three different scion treatment cultivars are represented in each of the days noted in this figure in order to demonstrate similarities in the whole plant transpiration curves across different scions within a given day. Variations in transpiration curves are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration.

† = scion / rootstock

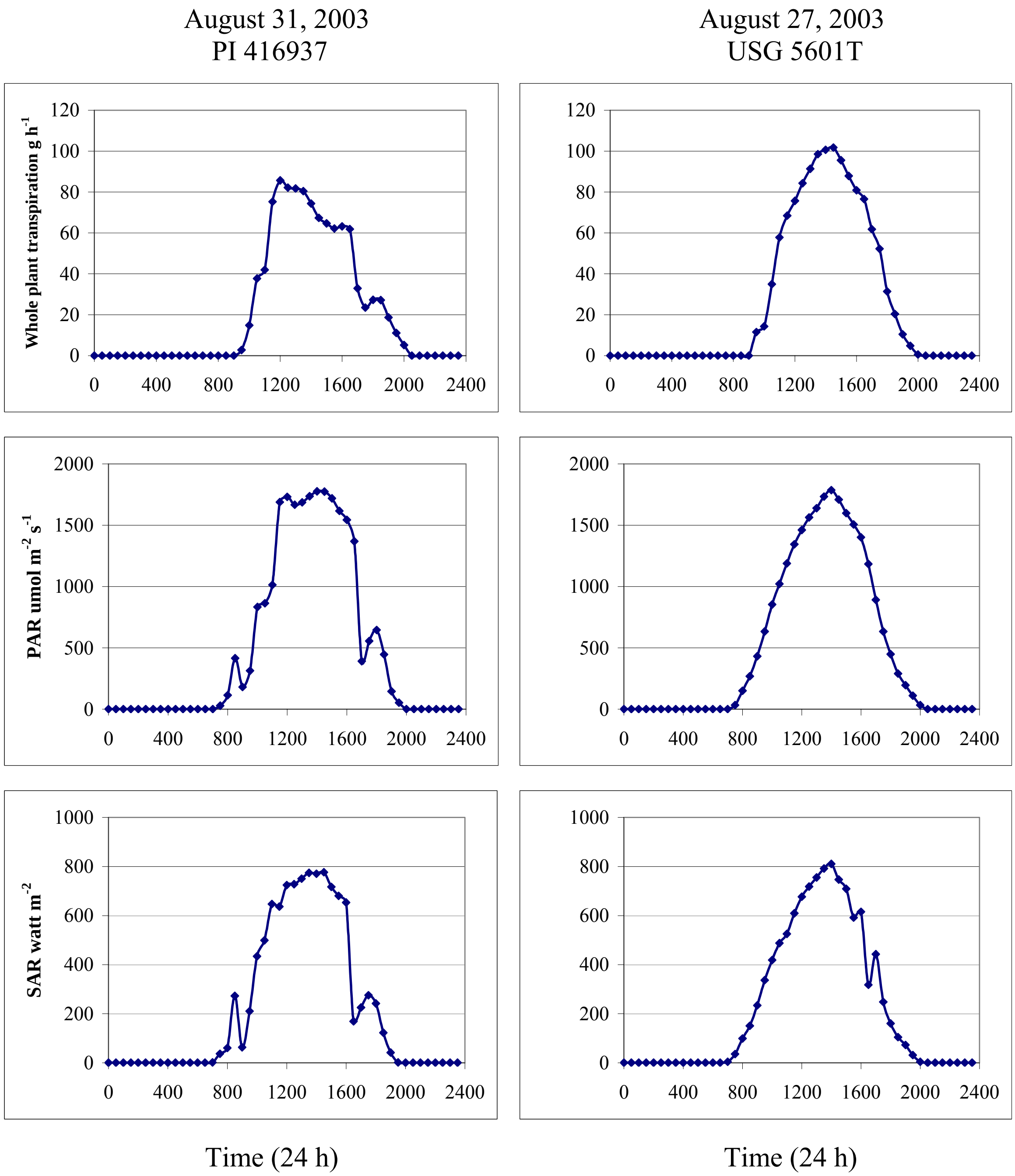

Figure 1.6. Whole plant transpiration rates, photosynthetically active radiation and solar radiation measurements of cultivars PI 416937 and USG5601T, recorded during two different 24 hour periods in 2003. The similarity in the curves within each day demonstrates the close relationship between PAR and transpiration. The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration.

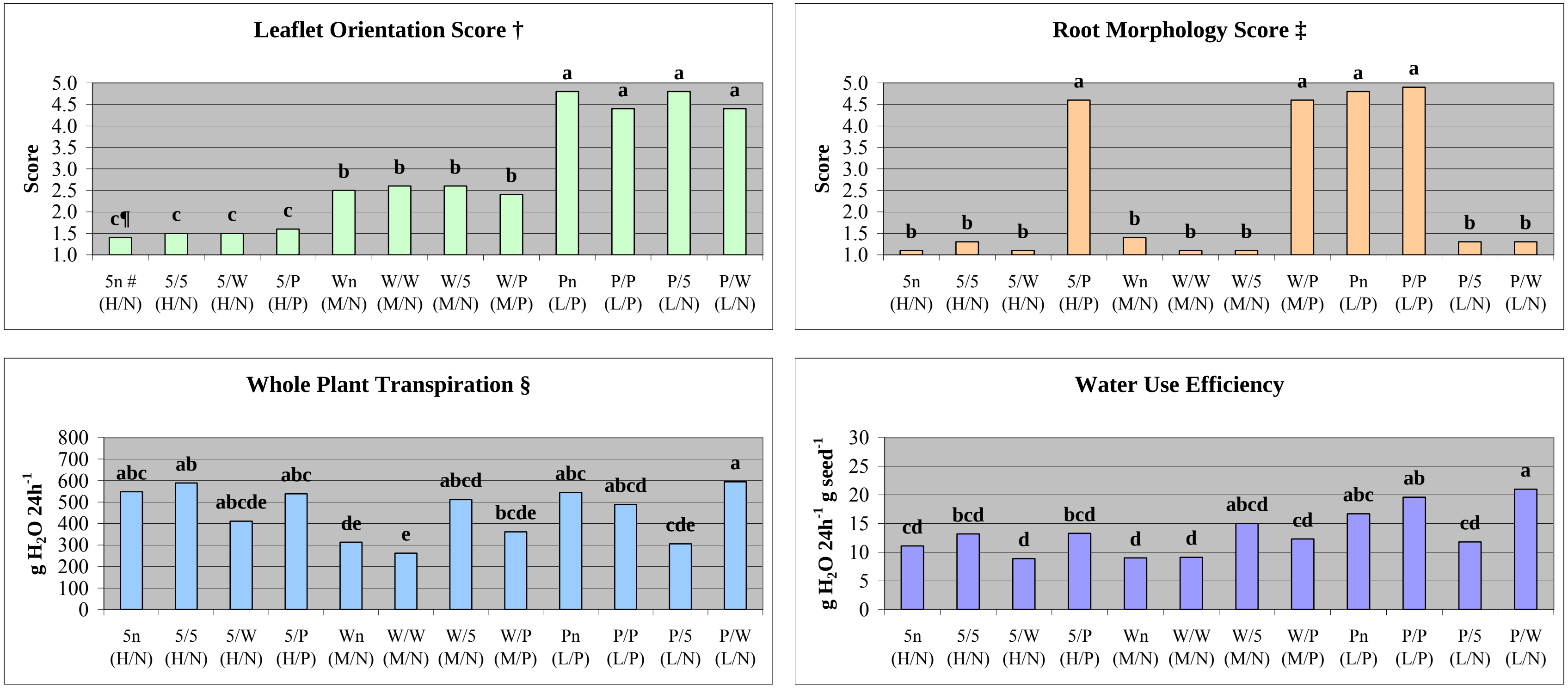

Figure 1.7. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 12 grafting treatments consisting of high, medium, and low leaflet orientation and normal or prolific root morphology evaluated in 2003 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.

¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

#; grafting treatments 5=USG 5601T, W=Williams 82, P=PI 416937; 5n denotes USG 5601T (non grafted),5/5 denotes USG 5601T scion/USG 5601T rootstock (self graft), 5/W denotes USG 5601T scion / Williams 82 Rootstock, etc. The relative class of the leaflet orientation of the scion (High, Medium, Low) and the relative class of the root morphology of the rootstock (Normal or Prolific) is denoted below the treatment designation i.e. (H/N) indicates that the grafting treatment scion had high leaflet orientation and normal root morphology.

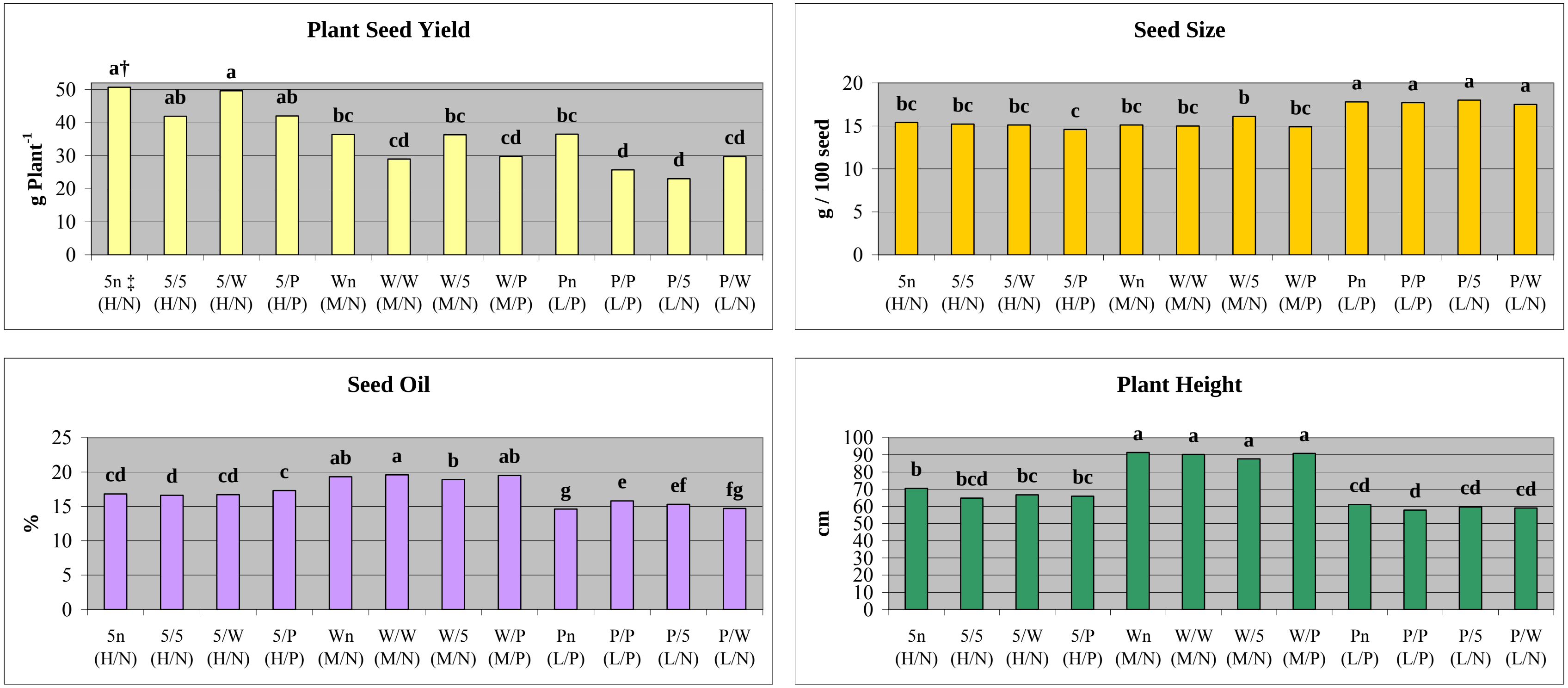

Figure 1.8. Comparison of plant seed yield, seed size, seed oil and plant height of 12 grafting treatments consisting of high, medium, and low leaflet orientation and normal or prolific root morphology evaluated in 2003 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

‡; grafting treatments 5=USG 5601T, W=Williams 82, P=PI 416937; 5n denotes USG 5601T (non grafted),5/5 denotes USG 5601T scion/USG 5601T rootstock (self graft), 5/W denotes USG 5601T scion / Williams 82 Rootstock, etc. The relative class of the leaflet orientation of the scion (High, Medium, Low) and the relative class of the root morphology of the rootstock (Normal or Prolific) is denoted below the treatment designation i.e. (H/N) indicates that the grafting treatment scion had high leaflet orientation and normal root morphology.

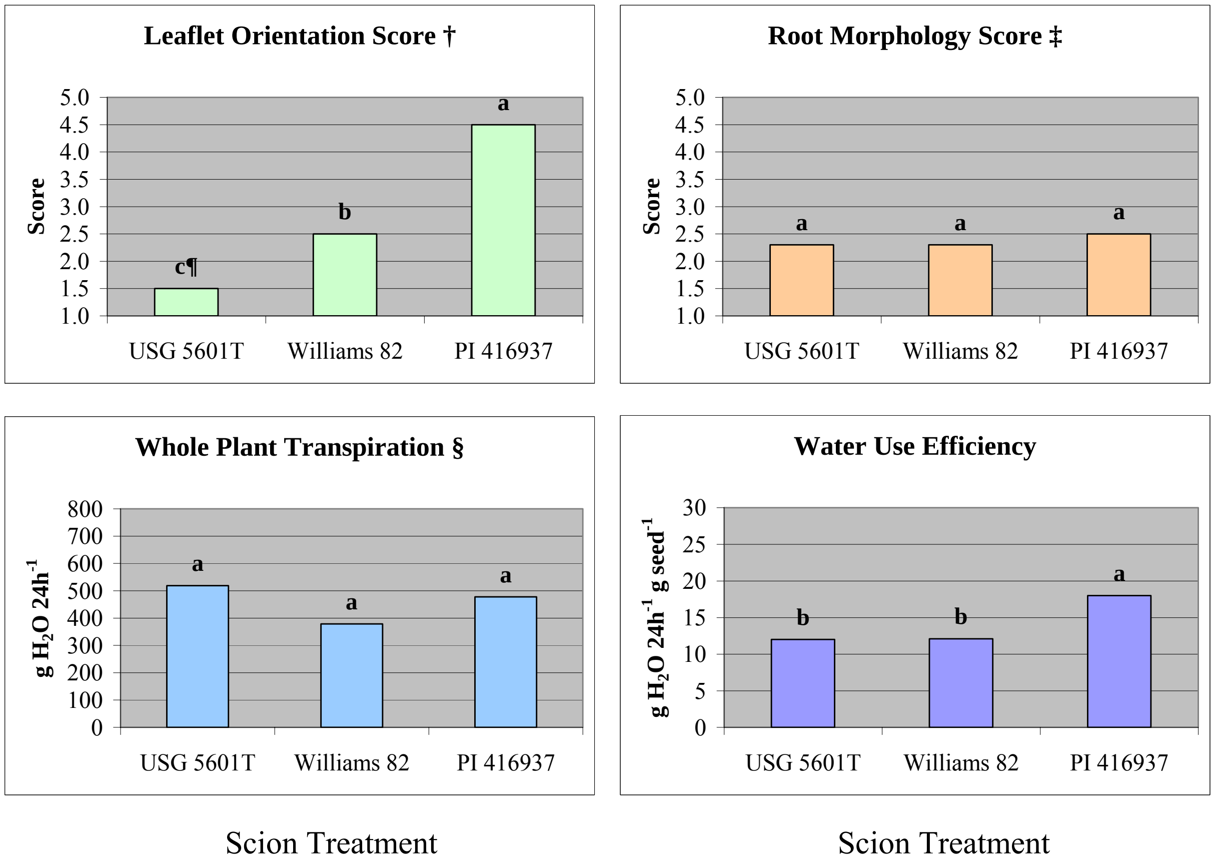

Figure 1.9. Comparison of scion treatment effects (averaged across rootstocks), on leaflet orientation, root morphology, whole plant transpiration, and water use efficiency evaluated in 2003 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.

¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

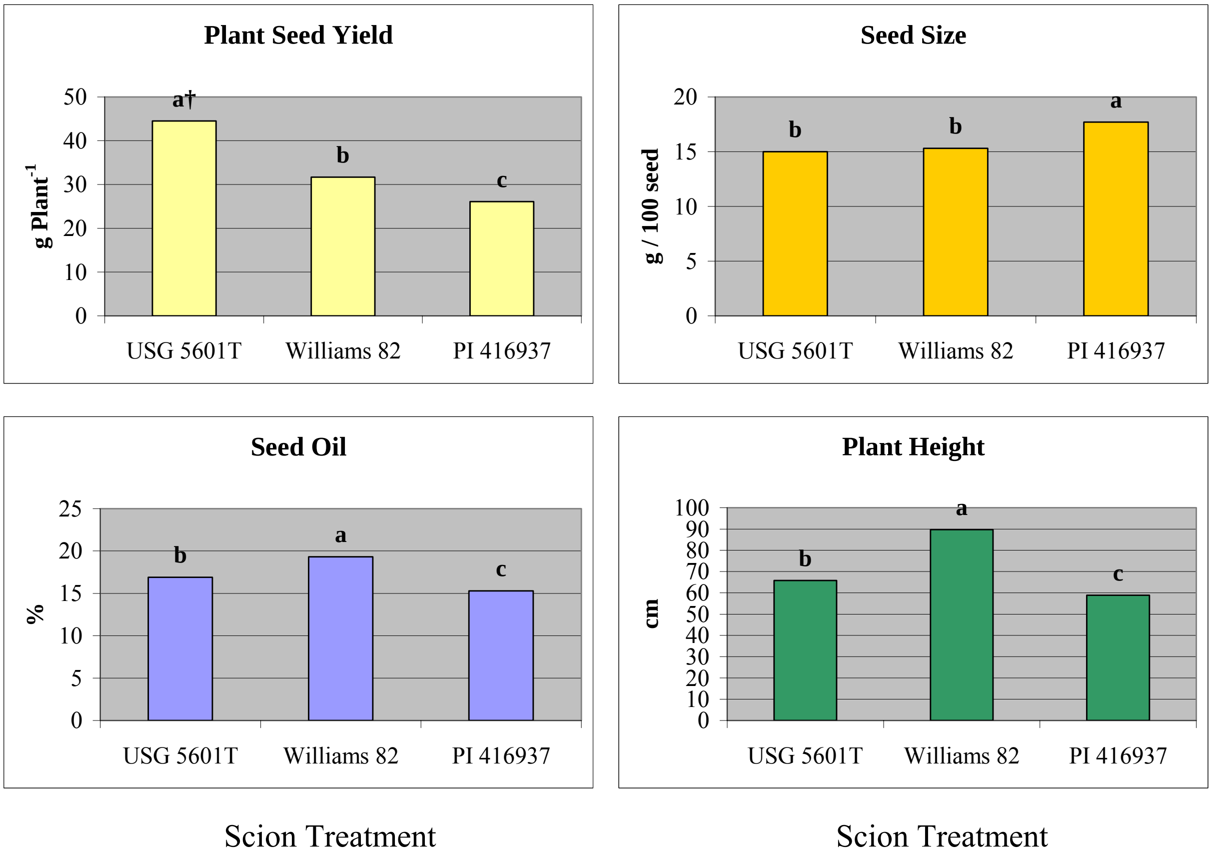

Figure 1.10. Comparison of scion treatment effects (averaged across rootstocks) on plant seed yield, seed size, seed oil, and plant height evaluated in 2003 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

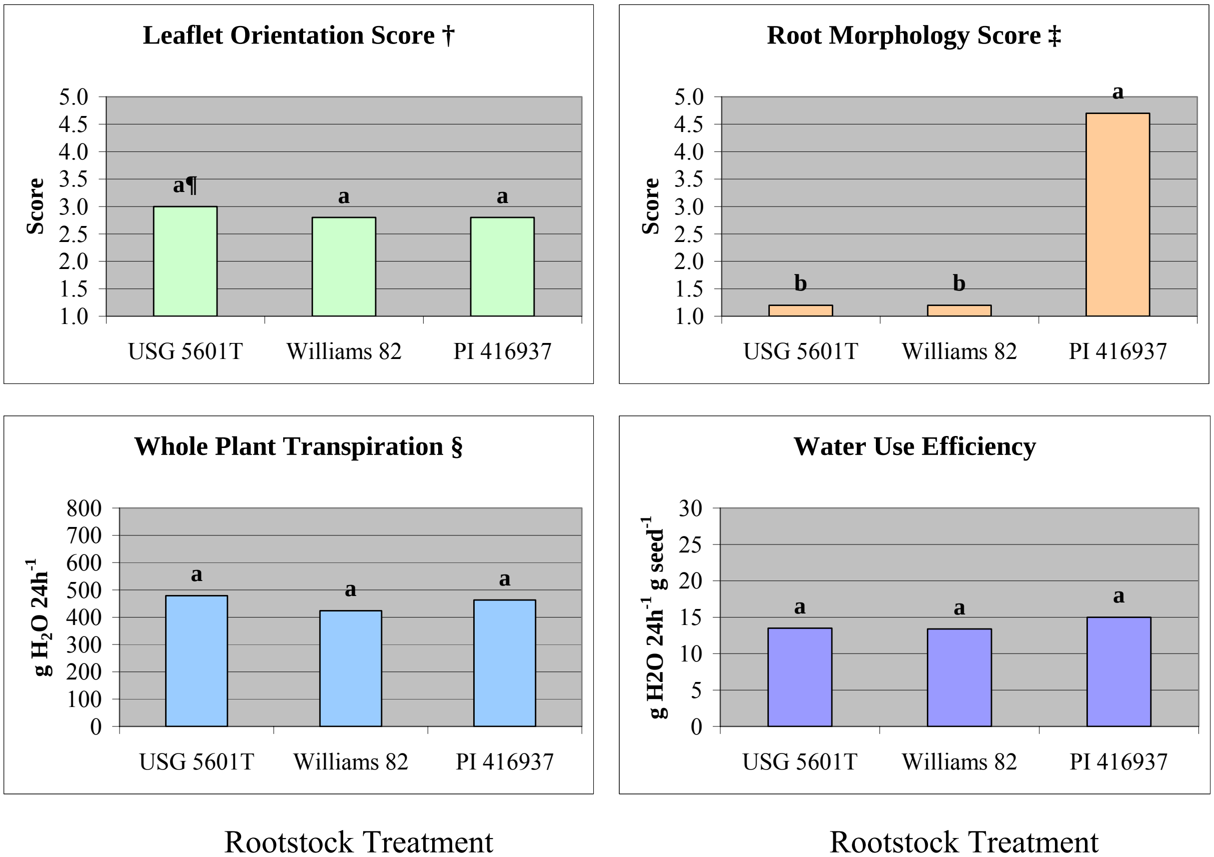

Figure 1.11. Comparison of rootstock treatment effects (averaged across scions) on leaflet orientation, root morphology, whole plant transpiration, and water use efficiency evaluated in 2003 at Knoxville, TN

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.

¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

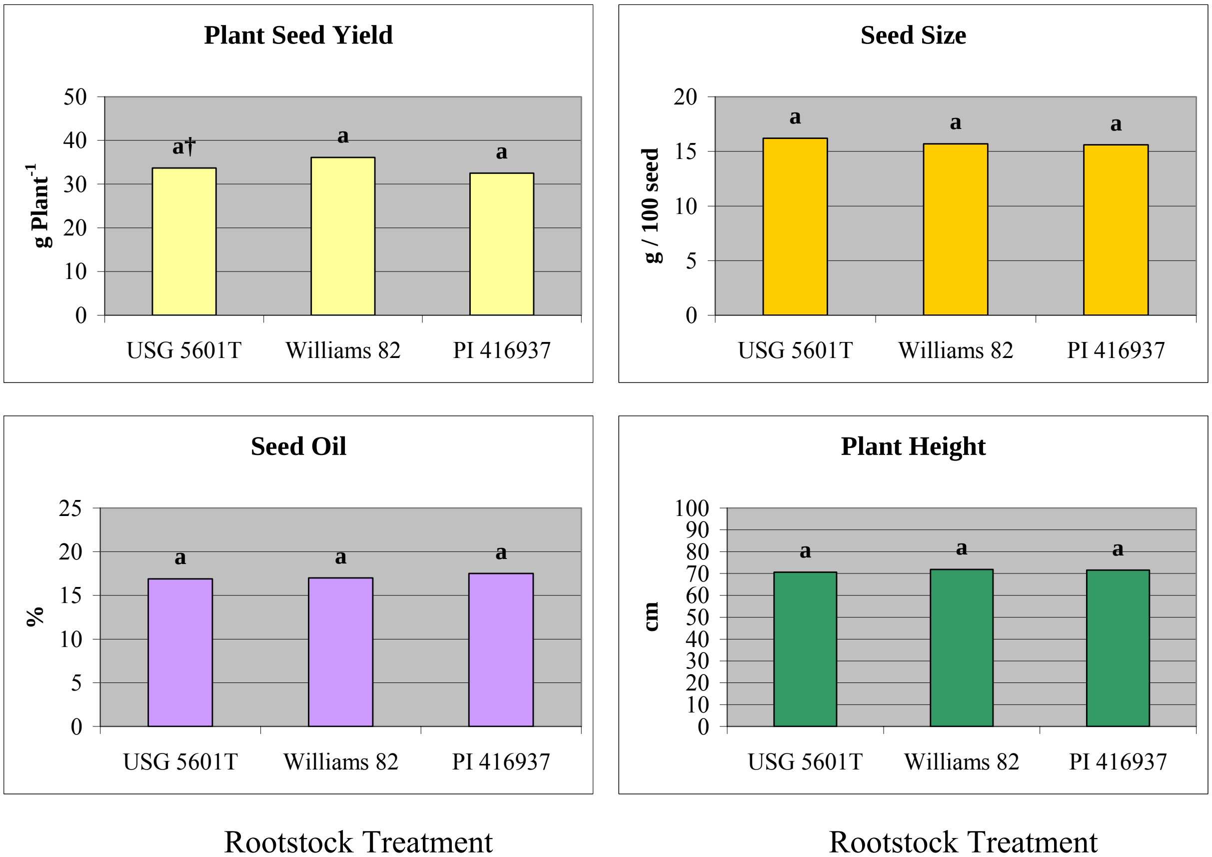

Figure 1.12. Comparison of rootstock treatment effects (averaged across scions) on plant seed yield, seed size, seed oil, and plant height evaluated in 2003 at Knoxville, TN.
† = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

APPENDIX B

Part I

Supplemental Tables

Table B.1.1 Differences in average whole plant transpiration, seed yield, water use efficiency, seed size plant height, seed protein and oil among 12 grafting treatments utilizing three soybean lines differing in leaflet orientation and root morphology.

Cultivar grafting treatment	Leaflet orientation / rootstock	Scion leaflet orientation	Rootstock root morphology	Whole plant transpiration	Seed yield	Water use efficiency §	Seed size	Plant Height	Seed Protein	Seed Oil
(Scion / Rootstock)	(type)	(score)†	(score)‡	(g H_20 24h^{-1}) §	(g plant^{-1})	(g H_2O 24h^{-1} g seed yield^{-1})	(g 100 seed^{-1})	(cm)	(%)#	(%)#
USG 5601T (non grafted)	High / Normal	1.4 c¶	1.1 b	548.1 abc	50.7 a	11.1 cd	15.4 bc	70.5 b	43.2 bc	16.8 cd
USG 5601T / USG 5601T	High / Normal	1.5 c	1.3 b	588.6 ab	41.9 ab	13.2 bcd	15.2 bc	64.8 bcd	43.3 bc	16.6 d
USG 5601T / Williams 82	High / Normal	1.5 c	1.1 b	410.6 abcde	49.6 a	8.9 d	15.1 bc	66.7 bc	43.1 bcd	16.7 cd
USG 5601T / PI 416.937	High / Prolific	1.6 c	4.6 a	538.6 abc	42.0 ab	13.3 bcd	14.6 c	66.0 bc	42.3 cde	17.3 c
Williams 82 (non grafted)	Medium / Normal	2.5 b	1.4 b	313.3 de	36.4 bc	9.0 d	15.1 bc	91.4 a	40.4 f	19.3 ab
Williams 82 / Williams 82	Medium / Normal	2.6 b	1.1 b	262.2 e	29.0 cd	9.1 d	15.0 bc	90.2 a	41.4 ef	19.6 a
Williams 82 / USG 5601T	Medium / Normal	2.6 b	1.1 b	512.3 abcd	36.3 bc	15.0 abcd	16.1 b	87.6 a	41.9 de	18.9 b
Williams 82 / PI 416.937	Medium / Prolific	2.4 b	4.6 a	361.3 bcde	29.8 cd	12.3 cd	14.9 bc	90.8 a	40.6 f	19.5 ab
PI 416.937 (non grafted)	Low / Prolific	4.8 a	4.8 a	544.8 abc	36.5 bc	16.7 abc	17.8 a	61.0 cd	44.5 a	14.6 g
PI 416.937 / PI 416.937	Low / Prolific	4.4 a	4.9 a	488.9 abcd	25.7 d	19.6 ab	17.7 a	57.8 d	42.8 bcd	15.8 e
PI 416.937 / USG 5601T	Low / Normal	4.8 a	1.3 b	305.6 cde	23.0 d	11.8 cd	18.0 a	59.7 cd	43.6 ab	15.3 ef
PI 416.937 / Williams 82	Low / Normal	4.4 a	1.3 b	594.8 a	29.7 cd	21.0 a	17.5 a	59.1 cd	43.9 ab	14.7 fg
	Pr>F $_{.05}$	<0.0001	<0.0001	0.0266	<0.0001	0.0095	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on two plants per treatment per date at R4-R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ on 15 Aug., 27 Aug., 31 Aug., and 9 Sept. 2003
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance
# = protein and oil reported on a dry weight basis

Table B.1.2. Comparison of whole plant transpiration, seed yield, water use efficiency, seed size, plant height, seed protein and oil of scion grafting treatments averaged by cultivar across rootstock treatments (non-grafted treatments excluded).

Scion cultivar across rootstocks	Leaflet orientation	Root morphology	Whole plant transpiration	Seed yield	Water use efficiency §	Seed size	Plant height	Seed Protein	Seed Oil
	(score)†	(score)‡	(g H_20 24h^{-1}) §	(g plant^{-1})	(g H_2O 24h^{-1} g seed yield^{-1})	(g 100 seed^{-1})	(cm)	(%)#	(%)#
USG 5601T	1.5 c¶	2.3 a	519.2 a	44.5 a	12.0 b	15.0 b	65.8 b	42.9 a	16.9 b
Williams 82	2.5 b	2.3 a	378.6 a	31.7 b	12.1 b	15.3 b	89.7 a	41.3 b	19.3 a
PI 416.937	4.5 a	2.5 a	477.6 a	26.1 c	18.0 a	17.7 a	58.9 c	43.4 a	15.3 c
$Pr>F_{.05}$	<0.0001	0.9717	0.1508	<0.0001	0.0127	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
§ = measurements taken on two plants per treatment per date at R4-R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ on 15 Aug., 27 Aug., 31 Aug., and 9 Sept. 2003.
¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance.
# = protein and oil reported on a dry weight basis.

Table B.1.3. Comparison of whole plant transpiration, seed yield, water use efficiency, seed size, plant height, seed protein and oil of rootstock grafting treatments averaged by cultivar across scion treatments (non-grafted treatments excluded).

Rootstock cultivar across scions	Leaflet orientation	Root morphology	Whole plant transpiration	Seed yield	Water use efficiency §	Seed size	Plant height	Seed Protein	Seed Oil
	(score)†	(score)‡	(g H_20 24h^{-1}) §	(g plant^{-1})	(g H_2O 24h^{-1} g seed yield^{-1})	(g 100 seed^{-1})	(cm)	(%)#	(%)#
USG 5601T	3.0 a¶	1.2 b	478.9 a	33.7 a	13.5 a	16.2 a	70.6 a	42.9 a	16.9 a
Williams 82	2.8 a	1.2 b	424.0 a	36.1 a	13.4 a	15.7 a	71.9 a	42.8 a	17.0 a
PI 416.937	2.8 a	4.7 a	462.9 a	32.5 a	15.0 a	15.6 a	71.6 a	41.9 a	17.5 a
$Pr>F_{.05}$	0.9501	<0.0001	0.7638	0.5784	0.7382	0.4874	0.9768	0.1462	0.7848

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
§ = measurements taken on two plants per treatment per date at R4-R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ on 15 Aug., 27 Aug., 31 Aug., and 9 Sept. 2003.
¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance.
# = protein and oil reported on a dry weight basis.

Part II

Effects of Soybean Leaflet Orientation and Root Morphology on Transpiration, Yield and other Physiological Traits among Population Lines

Abstract

Drought is considered the most important abiotic stress that reduces global soybean yield and breeding for drought tolerance traits is a goal in many plant breeding programs. The objective of this study was to investigate the effects of two proposed drought tolerance traits, leaflet orientation and prolific rooting, both singly and in combination, on physiological traits and yield of soybean. Experiments were conducted across the state of Tennessee (USA) during the 2005, 2006, 2007, and 2008 growing seasons. Two hundred and ten $F_{4:6}$, $F_{7:8}$, $F_{7:9}$, and $F_{7:10}$ lines from the cross USG 5601T × PI 416937, which segregated for the two traits of interest, were evaluated for whole plant transpiration rates, single plant yield, biomass production, leaf area, seed size, leaflet transpiration, stomatal conductance, photosynthesis rates, photosynthetically active radiation (PAR), solar radiation (SAR), leaflet temperatures, leaflet orientation and root morphology scores at Knoxville, TN USA (35.89 lat., -83.96 long.). Whole plant transpiration was measured on two to four plants of each line in each year of the study using a Dynamax Flow 32 Sap Flow Monitoring System when the plants were in the active pod filling stages of growth (R4-R6). The amount of water transpired by the treatment in a 24 h period during seed fill was divided by the grams of seed produced by that plant in order to obtain estimates of water use efficiency. Replicated plots were also planted at Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan, TN (35.93 lat., -88.70 long.). All data were analyzed using SAS Proc Mixed with the soybean lines considered as fixed effects and all other effects considered random in order to obtain least squares means of traits for each line for each year/location. Least squares means of each line were then used in the correlation and phenotypic class analyses. High leaflet orienting parental line, USG5601T, exhibited

lower mid-canopy reductions in rates of leaflet PAR, temperature, transpiration, stomatal conductance, and photosynthesis than the low leaflet orienting parental line, PI 416937. Frequency distributions of population lines for leaflet orientation and root morphology scores approximated normal distributions suggesting that the two traits are polygenic in nature. Light penetration into middle canopy was significantly higher for population lines which exhibited high leaflet orientation than for those that exhibited medium or low leaflet orientation. Leaflet temperatures of high leaflet orienting population lines averaged 5.2°C cooler than leaflets exposed to full direct sunlight. Low leaflet orienting lines in this population were associated with better water use efficiency ($r=-0.28$, $p=0.04$), later maturity ($r=0.34$, $p=0.01$), larger seed size ($r=0.30$, $p=0.03$), higher leaf area ($r=0.42$, $p=0.002$), lower seed oil ($r=-0.47$, $p=0.0003$), higher seed protein ($r=0.18$, $p=0.01$), and higher biomass accumulation ($r=0.42$, $p=0.002$). There were no significant associations between lower leaflet orientation and whole plant transpiration or yields however, there were patterns that suggested a general relationship to higher transpiration and yield. High leaflet orienting lines were associated with the same traits but in the opposite manner. Many of these phenotypic trait associations are consistent with the parental phenotype suggesting some degree of linkage. Leaflet orientation and root morphology were found to be significantly correlated ($r=0.33$, $p=0.02$). Population lines exhibiting prolific rooting were associated with higher transpiration rates ($r=0.29$, $p=0.03$), higher yield ($r=0.29$, $p=0.03$), later maturity ($r=0.49$, $p=0.0002$), lower seed oil ($r=-0.28$, $p=0.04$), higher leaf area ($r=0.43$, $p=0.001$), higher biomass accumulation ($r=0.37$, $p=0.006$), and higher upper canopy leaflet photosynthesis rates ($r=0.49$, $p=0.0002$). A single correlation between prolific rooting and better water use efficiency ($r=-0.33$, $p=0.01$) was detected, however the strength of the data was not compelling as other associations between these traits were not significant and were not always in the same direction in the correlation and phenotypic class analyses. Normal rooted lines were associated with the same traits but in the opposite manner. Many of the phenotypic trait associations were consistent with the parental phenotype again suggesting some degree of linkage. No significant differences were detected between the combination classes which represent the extreme phenotypic leaflet orientation and root morphology combinations of H/N, L/P, H/P, and L/N for whole plant transpiration, single plant yield, plot yield, water use efficiency, or seed protein content indicating that no additive gain was realized. This lack of an additive or reductive response could be due to the correlation between leaflet orientation and root morphology which was detected in this study. Regarding associations between the leaflet orientation and root morphology traits and whole plant transpiration and yield, it is likely that leaflet orientation (rather than root morphology) is responsible for detected differences since other studies involving grafted plants and isogenic pairs found no effect on these traits when comparing normal roots to that of prolific roots. Significant differences were detected in some single year analyses between the combination classes which represent the extreme phenotypic combinations of H/N, L/P, H/P, and L/N for seed size, maturity, plant height, lodging, seed oil content, dry weight biomass accumulation, and leaf area. However, the patterns observed only reflected the previously described trends of lower leaflet orientation being associated with larger seed size, later maturity, slightly increased plant height and lodging, lower seed oil content, higher biomass accumulation, and higher leaf area. The root morphology phenotypes seemed to have little to no effect on the expression of these traits when analyzed as combined phenotypic classes.

Chapter I

Introduction

The concept of ideotype breeding involves identifying morphological traits that affect overall fitness, desirability and yield in a positive manner and then using those traits to assist in selection of superior performing genotypes (Donald, 1968). Ideotypes will vary depending on the species, environment, and overall goals of the breeding project. Many early studies involving this concept have involved canopy and root characteristics. Progress under this concept has been slow as many of the traits are complex and controlled by many genes. The effects of these traits on yield can be small and therefore it is often difficult to detect a causal relationship. Additionally morphological traits may be linked to undesirable traits (Hamblin, 1993).

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress as it adversely affects total world soybean yield by approximately 40% (Pathan et al., 2007). Consequently, drought tolerance is a highly sought after trait in soybean cultivars. Drought tolerance is a complex response and is conditioned by the interaction of several genetic traits of the plant to environmental conditions (Chaves et al., 2003). Knowledge of these trait processes is needed not only to understand plant resistance to drought stress but also to improve crop management and breeding techniques.

Many of the traits that are attributed to plant adaptation during drought such as phenology, root size and depth, and hydraulic conductivity are associated with plant development and structure and are constitutive rather than stress induced. A considerable part of plant resistance to drought is the ability to dissipate or avoid excess radiation. The nature of the mechanisms responsible for leaf photoprotection, especially those related to thermal dissipation and oxidative stress are therefore of great interest. A desirable plant type would be one that could endure drought conditions while maintaining a higher level of productivity by avoiding tissue dehydration and sustaining tissue water potential and photosynthesis as high as possible. Adaptive traits which condition dehydration avoidance include those which minimize excessive water loss and maximize water uptake. Water loss may be reduced by reducing light absorbance via steep leaf angles. Water uptake may be maximized by increasing the rooting volume and/or depth (Chaves et al., 2003). Two potential traits of interest are therefore leaflet orientation and root morphology. Leaflet orientation addresses the need to reduce water loss and root morphology addresses the ability to maximize water uptake.

Leaflet orientation

Many species of plants are capable of leaf movements in response to external stimuli (Ehleringer and Forseth, 1980). Leaf movement in response to light, known as heliotropism, can be classified as either diaheliotropic (light seeking) or paraheliotropic (light avoiding). Plants exhibiting diaheliotropism orient the plane of the leaf blade perpendicular to incident light rays, while plants exhibiting paraheliotropism orient the plane of the leaf blade parallel to incident light rays. Soybean exhibits both diaheliotropic and paraheliotropic movements, with the degree of movement being dependent on genotypic response (Wofford and Allen,

1982) and various levels of environmental stimuli (Ehleringer and Forseth, 1989; Rosa and Forseth, 1995).

Paraheliotropism and diaheliotropism provide a means by which the plant can alter the arrangement of its leaves in order to gain maximum benefit from the environment. Advantages of changing leaf angle and light absorbance include increased total canopy light interception (Kawashima, 1969 a,b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000), increased photosynthetic efficiency (Pichard and Forseth, 1988; Gamon and Pearcy, 1989; He et al., 1996; Kawashima, 1969a,b; Arena et al., 2008), and , increased yield (Wang et al., 1995; Chang and Tagumpay, 1970; Mickelson et al., 2002; Pendleton et al., 1968; Pepper et al., 1977). Leaflet orientation can also reduce leaf temperature (Pichard and Forseth, 1988; Gamon and Pearcy, 1989; Wang et al., 1993; Forseth and Teramura, 1986; Rosa et al., 1991; Kao and Forseth, 1992; Paris, 1997; Bielenberg et al., 2003; Yu and Berg, 1994; Rosa and Forseth, 1995; Isoda and Wang, 2002; Arena et al., 2008; He et al., 1996; Stevenson and Shaw, 1971; Isoda et al., 1992, 1993; Isoda and Tomagae, 2003) which can reduce excessive transpiration rates (Pichard and Forseth, 1988; Bielenberg et al., 2003; Kao and Forseth, 1992; Yu and Berg, 1994; Isoda and Wang, 2001, 2002; Wien and Wallace 1973; Shackel and Hall, 1979; Meyer and Walker, 1981; Berg and Hsiao, 1986; Forseth and Teramura, 1986; Berg and Heuchelin, 1990). Additionally paraheliotropism can reduce photoinhibition (Hirata et al., 1983; Prichard and Forseth, 1988; Rosa et al., 1991; Rosa and Forseth, 1995; He et al., 1996; Jiang et al., 2006; Kao and Tsai, 1998), and increase water use efficiencies (Pichard and Forseth, 1988; Rosa et al., 1991; Kao and Forseth, 1992; Bielenberg et al., 2003; Kao and Tsai, 1998). In soybean, this phenomenon may be a mechanism to reduce water loss while maintaining some level of productivity as reported by

Meyer and Walker (1981). Paraheliotropic leaf movements reduce transpirational water loss by lowering light interception of leaves, consequently improving water status and lowering leaf temperature.

Research conducted at the University of Tennessee demonstrated that soybean cultivars differ in their ability to orient leaflets during the course of the day (Wofford and Allen, 1982). Most cultivars exhibit high leaflet orientation (paraheliotropism) and move their leaves during the course of the day such that the leaves have maximum exposure to the sun in the early and late parts of the day, but during mid-day the leaves are oriented parallel to sunlight such that the surface of the leaves has minimum exposure to the sun. A lesser number of cultivars exhibit low leaflet orientation where the leaf surface remains relatively flat and changes little relative to the position and intensity of sunlight, even during the mid-day period of highest irradiance. These "low leaflet orienting" types are therefore relatively less paraheliotropic. In a study of the cultivar Essex (high leaflet orientation) and Dare (low leaflet orientation), the two cultivars produced about equal yields; however Essex used about one-half the amount of water as Dare during the growing season (Paris, 1997).

In work with soybean, Lugg and Sinclair (1981) found that upper leaflets of the canopy maintained a higher net photosynthetic rate per unit leaf area than did the lower leaflets. This seemed to be mostly due to shading, as the lower leaves were found to have photosynthetic rates similar to upper canopy leaves when unshaded. Kawashima (1969 a,b) found that soybean leaflets exhibiting paraheliotropism in the upper canopy allowed light to penetrate more deeply into the canopy, increasing photosynthetic output of the lower leaves, thus allowing total photosynthetic efficiency of the plant to be improved. Vertical leaf angles decrease the amount of solar radiation intercepted by the leaf. However photosynthetic rate response in plants to solar radiation is nonlinear and saturates below the intensity of direct ambient sunlight (van Zanten et al., 2010). Soybeans are reported to maximize their photosynthetic rates at less than one-third the amount of full sunlight according to Beuerlein and Pendleton (1971). Vertical leaflet orientation increases overall photosynthesis by allowing the upper canopy leaves to continue to photosynthesize under lower than ambient sunlight while also allowing lower canopy leaves to contribute at an increased rate (van Zanten et al., 2010).

Kao and Tsai (1998) studied leaf movements in three soybean species and found that paraheliotropism seemed to enhance water use efficiency and decrease the risk of photoinhibition in plants under water stress. Grant (1999) found that soybean plants that exhibit paraheliotropism are able to reduce UV-B irradiance in contrast to plants that do not orient leaflets. Ikeda and Matsuda (2002) studied photosynthetic efficiency differences in soybean leaves which were restrained from orienting versus naturally orienting. Their results indicated that paraheliotropic leaflet movements are an adaptation which optimizes net leaflet photosynthesis.

Isoda et al (1992, 1993) found that the paraheliotropic movements of soybean leaflets regulate light interception and reduce leaf temperature. Isoda and Wang (2002) studied leaf temperature and transpiration rates of cotton versus soybeans and found that soybeans were able to reduce leaf temperatures and transpiration rates. This was attributed to the soybean cultivars ability to orient its leaves in a paraheliotropic manner. In a study involving restrained and unrestrained soybean leaflets, Isoda and Tomagae (2003) found differences in temperature of up to 5.5° C between restrained and unrestrained leaflets of the same soybean cultivar.

Chang and Tagumpay, (1970) found that rice plants with erect leaves were correlated with higher yields while plants with drooping leaves were correlated with lower yields. Increased yields of maize hybrids have been associated with vertical leaf angle which allow more light penetration into the canopy (Mickelson et al., 2002; Pendelton et al., 1968; Pepper et al., 1977). Similarly leaflet orientation in soybeans has been related to increased light interception and yield potential (Shaw and Weber, 1967; Wang et al., 1995). However, Isoda and Tomagae (2003) compared biomass and seed yields of a highly orienting soybean cultivar which had its upper canopy leaves restrained from flowering to harvest in contrast to the same unrestricted cultivar. The study detected no differences in biomass or seed yields between the forced "low orienting" treatment and the "high orienting" control. There were also no differences detected in photosynthetic efficiencies or photoinhibition which may have been influenced by genotypic and/or environmental effects noted in the study as the results are contrary to previous research on the photosynthetic and photoprotective advantages of leaflet orientation (Shaw and Weber, 1967; Pichard and Forseth, 1998; Ikeda and Mastuda, 2002; Wang et al., 1995; Jiang et al., 2006; Hirata et al., 1983; Rosa et al., 1991; Rosa and Forseth, 1995; Kao and Tsai, 1998).

Root Morphology

Development of breeding lines that have superior root systems may be an effective way to stabilize crop yields in drought-prone regions (Chaves et al., 2003; Kell, 2011). The ability of plants to resist drought has been found to be proportional to the density and extent of root development (Quizenberry, 1982). More expansive root architecture also allows plants to exploit soil mineral resources which may aid in increased nutrition, drought tolerance and yield (Lynch, 1995). A deeper and more expansive root system may allow soybean plants to efficiently access more soil area and thus more soil moisture (Pathan et al., 2007, Taylor, 1980). This might increase the ability of soybean plants to uptake water in drought stressed environments.

Significant variation for root size and morphology has been found in soybean (Quizenberry, 1982; Howard, 1980). Boyer et al. (1980) found that more recently developed, higher yielding soybean lines had lower mid-day water deficits and larger root densities than older, lower yielding cultivars. Garay and Wilhelm (1983) found that isolines of the soybean cultivar Harosoy which had greater root density, explored deeper into the soil profile and extracted more water during drought stress than the normal isoline. Jin et al. (2010) reported that a group of higher yielding soybean lines tended to have greater biomass, root mass and rooting depth than a group of lower yielding lines.

A soybean plant introduction line from Japan, PI 416937 (Houjaku Kuwasu), which exhibits significant drought and aluminum tolerance (Goldman et al., 1989; Sloane et al., 1990; Hudak and Patterson, 1995) has been the focus of several researchers over the past 20 years. This soybean line has also been characterized as possessing an extensive fibrous-like prolific root morphology which differs from the normal tap root of most soybeans (Hudak and Patterson, 1995; Pantalone et al., 1996a, 1999). Several studies have indicated the unique rooting morphology of PI 416937 as a major component of its ability to tolerate drought (Hudak and Patterson, 1995, 1996; Chipman et al., 2001). The prolific rooting morphology of the PI has been shown to support increased numbers of nitrogen fixing nodules (Pantalone et al., 1996a; Patterson and Hudak, 1996) and enhanced nitrogen fixation (Marlow, 1993) which may contribute to drought tolerance. The PI root system has also been shown to penetrate and continue to grow through hard soil layers that were impenetrable to other cultivars (Busscher et al., 2000). In addition to its root morphology, studies have indicated that PI 416937 may also tolerate drought by means of its osmotic regulation which appears to be somewhat different than that of other soybean cultivars. Fletcher et al. (2007) reported that PI 416937 demonstrated the ability to limit its transpiration rate under conditions of vapor pressure deficits associated with low humidity. Other genotypes continued to increase transpiration rates under increasing vapor pressure deficits. This contributes to the explanation of decreased soil desiccation by PI 416937 plants observed by Hudak and Patterson, (1996) and King et al. (2009).

Water Use Efficiency

Water use efficiency of crop plant can be improved by selection for improved transpiration efficiency and harvest index (Turner, 1993). Purcell (2006) stated that the main tenets of crop physiology are that crop mass and yield are proportional to the cumulative amount of light intercepted and to the amount of water transpired by the crop during a season. Research indicates this to be true although the relationships may be more curvilinear than previously perceived. Edwards et al. (2005) found that although yield continued to increased with cumulative intercepted photosynthetically active radiation through 1100 MJ m^{-2}, 90% of maximum soybean yield can be obtained by intercepting 605 MJ m^{-2}. Similarly, Purcell et al. (2007) found that while soybean yield continued to increase with cumulative transpiration through 750 mm of soil profile water, 90% of the maximum yield could be obtained by transpiring 444 mm. This is encouraging for researchers who wish to improve soybean water use efficiencies as it indicates genotypes may exist, or can be developed, that regulate transpiration and light interception in such a manner as to maximize yield while using only as much water as needed. Identification of these types of plants and their associated traits would be of great interest to plant breeders and other researchers.

The objective of this research was to investigate the effects of leaflet orientation and prolific rooting, both singly and in combination, on transpiration, seed yield, water use efficiency, leaflet temperature, leaflet photosynthesis rate, canopy light penetration, biomass and other physiological traits of soybean.

Chapter II

Materials and Methods

Experiments were conducted across the state of Tennessee (USA) during the 2005, 2006, 2007, and 2008 growing seasons using $F_{4:6}$, $F_{7:8}$, $F_{7:9}$, and $F_{7:10}$ population and parental lines in order to evaluate the effects of leaflet orientation and root morphology on leaflet temperature, canopy light penetrance, transpiration, stomatal conductance, photosynthesis, yield, water use efficiency, biomass accumulation, leaf area, and other agronomic traits in soybean.

In the summer of 2002, 28 potential parental cultivars were planted at Knoxville, TN USA (35.89 lat., -83.96 long.) on an Etowah silt loam soil (fine-loamy, siliceous, semiactive, thermic, Typic Paleudult) and evaluated for leaflet orientation and root morphology differences. Twelve crosses were initiated July, 2002 in an attempt to create populations which contained significant and visually detectable levels of segregation for the two traits. The F1 seed of these crosses were grown in Costa Rica (Semillas Olson S.A., Costa Rica) during the months of November 2002 to April 2003 and evaluated for purity and correctness using the traits of flower and pubescence color. The F2 populations were grown during May to October, 2003 at Knoxville on an Etowah silt loam soil (fine-loamy, siliceous, semiactive, thermic, Typic Paleudult) at which time the population USG 5601T × PI 416937, which contained the desired leaflet orientation and root morphology segregation patterns, was chosen for further study. USG 5601T is a recently released (Pantalone et al., 2003) high yielding, maturity group V, determinate cultivar that exhibits high leaflet orientation and typical tap root morphology. PI 416937 is a maturity group VI, determinate plant introduction that exhibits low leaflet orientation and prolific fibrous-like, root morphology (Pantalone et al., 1999) (Figs. 2.1, 2.2). Nine hundred and fifty six F2 plants were harvested and threshed separately at maturity using an Almaco BT-14 belt thresher (Almaco, Nevada, IA). F3 plants were advanced to the F4 generation by modified single seed descent (Brim, 1966) utilizing a winter nursery location in Homestead, FL (27 Farms, Homestead, FL) from November 2003 through April 2004. F3:4 generation lines were planted and evaluated for leaflet orientation at Knoxville during the 2004 growing season. Two hundred and ten F3:4 lines were selected for this study at that time. The selection criteria used was somewhat random with attempts to ensure that all maturity groups and leaflet orientation phenotype extremes were represented. No evaluation for root morphology segregation among lines was conducted at that time. Single plants were selected from each of the 210 F3:4 lines and advanced to the F4:5 generation, via progeny rows, at the winter nursery location in FL from November 2004 through April 2005. The F4:6 lines were grown and evaluated at Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan (35.93 lat., -88.70 long.), Tennessee in 2005. Due to resource and equipment limitations, 54 of these 210 lines were selected for further study based on their leaflet orientation and root morphology phenotypes. Additionally, 31 of the 210 lines developed from this population exhibited substantial shattering similar to the PI 416937 parent. Those lines which exhibited severe shattering were avoided from selection for further study since this trait would make accurate seed yield evaluations impractical. The selected lines were bulk advanced to the F4:7 generation and used for replicated plot yield trials in 2006. Additionally, the two F4:6 plants from each of the lines which had been evaluated for whole plant transpiration in 2005 and selected as part of the 54 lines for further study, were each advanced via progeny rows to the F6:7 generation at the FL winter nursery location where they were harvested as single plants and returned to Knoxville. The resulting 54 F7:8 lines were grown and evaluated in Knoxville for physiological and agronomic traits during the 2006, 2007 and 2008 growing seasons. There remained six lines in this study that exhibited varied levels of shattering depending on the location and year. Yield data that seemed significantly impacted by shattering were excluded from the analyses.

Leaflet orientation score for each plot was taken on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining and angle parallel to the horizontal plane (Figure 2.1). Three replications of leaflet orientation scores were taken on 9 August, 24 August, and 8 September in 2005 and on 16 August, 25 August and 16 September in 2006 concurrent with the measurement period of whole plant transpiration. Leaflet orientation was rated between the hours of 1300 and 1500 for each replication as this is the period of the day in which the differential leaflet orientation was at its highest (Wofford and Allen, 1982).

Root morphologies were scored on three replications of hill plots consisting of lines and parents via a soil inverter in 2005 and 2006 following the method described by Pantalone et al. (1996a). Ratings were obtained when plants were in the R4 to R6 stage of growth on 2 September, 2005 and 25 August, 2006. Hill plots were planted in a slightly sandy, very friable Staser Silt Loam soil (fine-loamy, mixed, active, thermic, Cumulic Hapludoll) which facilitated soil removal and evaluation. Root morphology scores were based on a visual rating scale of 1 to 5 with 1 a normal tap root with few lateral roots and 5 being a prolific root mass with many fibrous-like lateral branching roots (Figure 2.2).

Leaflet temperatures were measured on random soybean leaves within the set of 54 lines with a Raytek model ST20 Pro infrared thermometer (Raytek Corp., Santa Cruz, CA) held at a distance of four to six inches from the leaf surface. Temperature measurements on leaflets which were exposed to full, partial or shaded sunlight exposure were taken in 60 and 20 replications in 2007 and 2008, respectively. Additionally, temperatures measurements were collected on leaflets which differed in the amount of sunlight exposure and their position in either the upper or middle plant canopy on 30 and 10 replications in 2007 and 2008, respectively. Measurements in 2007 were taken on 17 September between the hours of 1245 and 1530. Measurements in 2008 were taken on 20 September between the hours of 1420 and 1535.

In order to obtain measurements of potential differences in light penetration into the middle plant canopy, PAR measurements above and at mid-canopy were obtained from soybean lines differing in leaflet orientation with a Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX) and a model SF40 Decagon Sunflec Ceptometer (Decagon Devices Inc., Pullman, WA) in 2006 and 2008, respectively. Measurements in 2006 were taken on 16 September between the hours of 1448 and 1515 and included 13 of the 54 population lines and the two parental genotypes which were divided into three classes of high (score of 1.0-2.3), medium (score of 2.4-3.0), and low (score of 3.0-5.0) leaflet orientation each of which contained five lines. Measurements in 2008 were taken on 17 September between the hours of 1245 and 1337 and included 12 of the 54 population lines and parental genotypes which were similarly divided into classes containing five high, four medium, and five low leaflet orienting classes. In 2006, five replicated measurements of each leaflet orientation class were collected, while in 2008, 12 to 15 replicated measurements were collected on each leaflet orientation class of high, medium, and low.

Differences between upper canopy and middle canopy leaflets of parental lines for PAR, temperature, transpiration, stomatal conductance, and photosynthesis levels were measured with the Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX) in 2006. Eight replicated measurements were collected on each parental line for each canopy position treatment on 6 and 8 September, 2006 between the hours of 1311 and 1523 and the hours of 1254 and 1341, respectively.

Whole plant transpiration rates were measured at Knoxville on several successive days at 30 minute intervals using the Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stages of growth (R4-R6). Although this measurement may not be representative of transpiration over the growing season, it is deemed important as it represents the period in which seed yield and seed quality constituents are developed and water use is at or near its peak (Wilson, 2004; Heatherly and Elmore, 2004). Consequently, this is also the approximate period when leaflet orientation values were found to be at their highest by Wofford and Allen (1982). The maximum capacity of the Dynamax Flow 32 Sap Flow Monitoring System was 32 sensors. Therefore, only 16 or eight genotypes could be measured during any single measurement period in 2005 and 2006, respectively. Dynamax model SGA9 Flow32 System Dynagauges were used to connect each plant to the system as the approximate 9mm diameter size of the Dynagauge would properly fit around the lower stem of the plants at the time of measurement. Each plant was marked with a durable tag for identification purposes later in the season. The stem diameters were measured and cleaned. The interior of the Dynagauge sensor was lubricated with a very thin film of Dow Corning 4 Electrical Insulating Compound (Dow Corning Corp, Midland, MI) and then placed around the stem in such a manner as to ensure that the thermocouples and heater strip of the sensor were in direct contact with the stem. The top and bottom of the sensor were sealed with Elmer's Poster Tack adhesive putty (Elmer's Products Inc., Westerville, OH). The sensor was wrapped with a sheet of Reflectix double reflective insulation (Reflectix Inc., Markleville, IN) measuring approximately 14 cm x 33 cm which provides two layers of insulation. The insulation was held in place by cable ties placed near the top, bottom and middle of the sensor such that the insulation was secure but with minimal pressure being applied to the stem. The control system, cables, battery, and solar panel (battery recharge) were mounted to a vertical cart with wheels for easier transportation within the field (Fig. 2.3).

In 2005 whole plant transpiration data were collected on two plants for each of the 210 population lines for two to four days depending on environmental conditions. Only two plants were measured due to equipment and resource limitations and the large number of lines evaluated in 2005. In 2006 the number of lines evaluated was reduced to 54 and the number of plants measured per line for whole plant transpiration was increased to four plants per line. The number of lines was reduced due to concerns of sampling size, data variation and potential data loss due to sensor malfunction, as well as resource and equipment limitations. The goal was to collect data from a 24 h period when the conditions were mostly sunny; therefore some measurements covered a longer period of time due to cloudy days after the system was installed on the plant material. Whole plant transpiration data (g H_2O $24h^{-1}$) from a single mostly sunny day from each set of measurements were used in this analysis. Data collected on other days were not utilized due to factors such as sensor malfunctions and/or environmental conditions. Whole plant transpiration data were collected between 2 August and 11 September, 2005 and 11 August and 15 September, 2006. In order to adjust the measurements for variations due to differing environmental conditions between days, each set of measurements included the parents.

The plants which were tagged and measured for whole plant transpiration were harvested and threshed at maturity using an Almaco BT-14 belt thresher (Almaco, Nevada, IA) in order to obtain single plant yields. The amount of water transpired by the treatment in a 24h period during seed fill was divided by the grams of seed produced by that plant in order to obtain an estimate of water use efficiency.

Plots in all experiments were seeded using a commercial planter (John Deere, Max Emerge, Moline, IL) equipped with plot cone seeding units (model CTS, Almaco, Nevada, IA). All plots were seeded at a density of approximately 3 cm apart into two row plots which were 6 m in length with 76.2 cm spacing between rows. In addition to plots at Knoxville which were used to measure physiological traits, additional research plots were planted in order to evaluate yield and agronomic traits such as shattering, seed protein and oil. These locations included Knoxville, Springfield, Spring Hill, and Milan, Tennessee. Average plot yields, seed protein and oil measurements were derived from data collected across these four locations in 2005 and 2006. All plots at Knoxville during 2004 – 2007 were planted on a Stasser silt loam soil (fine-loamy, mixed active, thermic Cumulic Hapludoll), while the 2008 plots were planted on an Etowah silt loam soil (fine-loamy, siliceous, semiactive, thermic, Typic Paleudult). Yield trial plots at Springfield were planted on a Dickson silt loam soil (fine-silty, siliceous, semiactive, themic Glossic Fragiudult).

Yield trial plots at Spring Hill were planted on a Maury silt loam soil (fine, mixed, active, mesic Typic Paleudalf). Yield trial plots at Milan, TN USA (35.93 lat., -88.70 long.) were planted on a Vicksburg silt loam soil (coarse-silty, mixed, active, acid, themic Typic Udifluvent) and a Falaya silt loam soil (coarse-silty, mixed, active, acid, themic Aeric Fluvaquent) in 2005 and 2006, respectively. Each yield trial entry was replicated two times at each location. Yield trial plots were harvested at all locations with an Almaco SPC 40 plot combine (Almaco, Nevada, IA). Protein and oil analysis was performed on a Foss Model 1229 NIR analyzer (Foss NIRSystems Inc., Laurel, MD).

In 2006, four plants from each of the 54 F7:8 lines were collected concurrently with the transpiration measurement in order to obtain values of leaf area and biomass. Entire plants, including roots, were removed from the field and immediately weighed. The plants were then defoliated and the leaf area measured using a Delta T area meter (Delta T Devices, Cambridge, England). All plant parts were then dried in a forced-air dryer at a temperature of 54.4°C for approximately 96 hours or until such time as the weight of the sample stabilized. Samples were then weighed in order to obtain estimates of dry weight biomass production.

Photosynthetically active radiation (PAR), solar radiation (SAR), and soil moisture were recorded at 30 minute intervals at the Knoxville field location using a Hobo® weather station equipped with H21-001 data logger, S-LIA-M003 PAR sensor,S-LIB-M003 pyranometer, and S-SMA-M003 soil moisture sensors (Onset Computer Corporation, Pocasset, MA).

In 2006, leaflet transpiration, stomatal conductance, photosynthesis, PAR and leaflet temperature data were obtained from an upper canopy leaflet exposed to full sunlight from each of the 54 F7:8 lines using the Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX). These data were collected on 8 September, 11 September, and 14 September between the hours of 1345 – 1707, 1355 – 1508, and 1226 – 1353, respectively. Additionally data were collected using this device on leaflets from different levels of the plant canopy for the parental lines and other population lines varing in leaflet orientation phenotypes.

All data were analyzed using SAS Proc Mixed with the soybean lines considered as fixed effects and all other effects considered random in order to obtain least squares means of traits for each line for each year and location. Least squares means with mean separation and average LSD values were obtained using the SAS macro written by Saxton (1998). Least squares means of each line were then used in the correlation analyses via SAS Proc Corr. In order to further analyze the effects of leaflet orientation and root morphology on measured traits, the least squares means data were used to separate the data into high, medium and low leaflet orientation classes as well as normal, intermediate and prolific root morphology classes. Additionally combination classes were formed which separated lines into nine classes based on their leaflet orientation and root morphology phenotypes. These least squares means data sets were analyzed using SAS Proc GLM as model effects were previously adjusted and only independent and dependent variables remained (SAS User Guide 9.1.3, 2006).

Chapter III

Results and Discussion

Parental phenotype evaluation

The phenotypic contrasts of the parental lines, USG 5601T and PI 416937, were evaluated over the two year period. USG 5601T exhibited significantly higher leaflet orientation, water use efficiency, plot yield, plant height, and seed oil content than PI 416937 (Tables 2.1, 2.2). PI 416937 exhibited prolific rooting as well as significantly higher whole plant transpiration, single plant yield, maturity, lodging, shattering, seed size and seed protein content. Dry weight biomass measurements were not significantly different. Single plant yield and plot yield differences between the parents are contradictory as USG 5601T was found to have significantly higher plot yields but lower single plant yields. It is known from previous research that USG 5601T is a recently released cultivar with high yield (Pantalone et al., 2003), while PI 416937 is a plant introduction with relatively low yields (Slone et al., 1990). This is confirmed by the plot yield in the current study. Single plant yields are not considered reliable for estimating overall yield potential when measured on a limited number of plants grown at typical production population densities (as was the case in this study) due to environmental variation and interplant competition effects (Fasoulas, 1981; Fehr 1987; Lulsdorf and McVetty, 1986; Pasini and Bos, 1990). In contrast these authors relate findings that by space planting relatively large numbers of single plant hill plots, interplant competition is minimized and results correlate reasonably well with plot yields. In part 1 of this dissertation it was found that when USG 5601T and PI 416937 non-grafted plants were planted further apart (15 cm) than the current study, USG 5601T single plant yields were significantly higher than PI 416937 and proportionally in agreement with larger row plot yields.

When comparing the upper and middle canopy leaflet levels of PAR, temperature, transpiration, stomatal conductance, and photosynthesis, both parental lines displayed decreases (Table 2.3, Fig. 2.4). However the high leaflet orienting parent, USG5601T, exhibited lower percentages of change for all measured traits than low leaflet orienting parent, PI 416937. These results agree with reports from other researchers regarding the photosynthetic and photoprotective advantages of paraheliotropic leaflet movements (Kawashima, 1969ab; Kao and Forseth 1992; Wang et al., 1995; Jiang et al., 2006). PAR levels at mid canopy for USG 5601T was an average of 292 umol m^{-2} s^{-1} higher than PI 416937. Upper canopy leaflet temperatures of USG 5601T were cooler by 1.4° C as compared to PI 416937. This small but statistically significant difference could be due to differences in leaflet irradiance due to leaflet orientation. PI 416937 exhibited significantly higher leaflet transpiration in the upper canopy leaves than USG 5601T. Leaflet transpiration reduction for USG 5601T between upper and middle canopy leaflets was 14.1% while leaflet transpiration of PI 416937 was reduced by 32.9% (Table 2.3, Fig. 2.4). Similarly, stomatal conductance was reduced by only 36.5% in the middle canopy leaves of USG 5601T while PI 416937 was decreased by 59.1%. PI 416937 exhibited significantly higher photosynthesis rates in the upper canopy leaves relative to USG 5601T. However, the reduction in middle canopy leaflet photosynthetic rates was 86.1% in PI 416937 while USG 5601T was only reduced by 59.2%. This resulted in the middle canopy leaflets of USG 5601T maintaining a significantly higher overall photosynthetic rate relative to the middle canopy leaflets of PI 416937. This is most likely due to the increased amount of sunlight that the high orienting parent, USG 5601T, allows to penetrate into the middle canopy. Thus USG 5601T was able to maintain equal to higher levels of all measured traits in middle canopy leaflets relative its own upper canopy leaflets and to the middle canopy leaflets of the lower orienting parental line, PI 416937 (Table 2.3, Fig. 2.4).

Leaflet orientation and leaflet temperatures

Leaflet temperatures of random population lines over the two year period (2007-2008) which, by virtue of paraheliotropic leaflet orientation, were exposed to partial sunlight were an average of 5.2°C cooler than leaflets exposed to full direct sunlight (Fig. 2.5). Leaflets which were shaded due to either their positioning in mid canopy or aspect to sunlight were 8.8°C and 3.6°C cooler than leaflets exposed to direct or partial sunlight, respectively (Table 2.4, Fig. 2.5). These differences in leaflet temperature due to sunlight irradiance were consistent, significant and maintained regardless of the leaflet's position in the plant canopy (Table 2.5) and are consistent with other research findings (Pichard and Forseth, 1988; Wang et al., 1993; Rosa et al., 1991; Paris, 1997; Bielenberg et al., 2003; Stevenson and Shaw, 1971; Isoda and Tomagae, 2003).

Leaflet orientation and sunlight penetration into lower canopy

Light penetration into middle canopy was significantly higher for population lines which exhibited high leaflet orientation than for those that exhibited medium or low leaflet orientation (Fig. 2.6). These results are consistent with other researchers that report higher levels of light penetration onto the middle canopy leaves among plant canopies exhibiting paraheliotropic leaflet orientation (Kawashima, 1969 a,b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000). Compared to high leaflet orienting lines, average PAR levels in the middle canopy of medium and low leaflet orienting lines were 32 and 41 umol m^{-2} s^{-1} lower, respectively in 2006 (Table 2.6). Similarly in 2008, high leaflet orienting lines allowed more PAR sunlight penetration into the middle canopy than the medium or low leaflet orienting lines. In the latter case this resulted in a 126 and 156 umol m^{-2} s^{-1} increase in PAR at middle canopy compared to medium and low leaflet orienting lines, respectively (Table 2.7, Fig. 2.6). These differences were consistent and significant in both years of measurement, although the magnitude of the differences was much lower in 2006. This may have been due to differences in sensitivities of the two different measuring devices available to the study at those times (Dynamax LCi Ps meter in 2006; Decagon sunflec ceptometer in 2008).

Leaflet orientation effects

The frequency distribution of leaflet orientation scores of lines developed and evaluated from this cross approximated a normal distribution, which suggests that the expression of this trait is polygenic in nature (Allard, 1960) (Fig. 2.7). The Shapiro-Wilk statistic for normality was ($W=0.98$, $pr<W=0.006$) for the 2005 year analysis of the 210 population lines. The phenotypic classes of high, medium and low leaflet orientation, formed by segregation of lines on the basis of their least squares mean leaflet orientation scores separated significantly when analyzed as a phenotypic class for leaflet orientation. This appears to confirm the separation procedures were successful in creating distinct phenotypic classes in both the 210 F4:6 lines and the 54 F7:8 lines (Tables 2.8, 2.9)

Transpiration in the soybean plants during the monitoring period appeared to begin at approximately 0800 h, reaching a peak at approximately 1500 h, and ceasing at approximately 2000 h (Fig. 2.8). These whole plant transpiration rates were assumed to be representative of each line and parent during the reproductive stages of R4 – R6. While transpiration curves differed in overall magnitude, they were somewhat similar in shape within a day of measurement. There were variations in the overall shapes of the transpiration curves between days due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration. The similarities observed between the transpiration curves and the PAR curves within each day demonstrates the close relationship between PAR and transpiration (Fig. 2.9). The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis and transpiration. These observations are similar to those reported previously for soybean by Gerdes et al.(1994).

No significant differences in whole plant transpiration were detected between the high and low leaflet orientation classes over the two year period (Table 2.9, Figs. 2.10, 2.11). There may have been a slight trend towards the high leaflet orienting class transpiring less than the low or medium leaflet orienting classes, but the difference was only significant between the medium and high leaflet orienting classes (Table 2.9, Fig. 2.11). This is somewhat consistent with previous research which indicates that paraheliotropic leaf movements lower both leaflet temperatures and leaflet transpiration (Pichard and Forseth, 1988; Bielenberg et al., 2003; Kao and Forseth, 1992; Yu and Berg, 1994; Isoda and Wang, 2001, 2002; Wien and Wallace 1973; Shackel and Hall, 1979; Meyer and Walker, 1981;

Berg and Hsiao, 1986; Forseth and Teramura, 1986; Berg and Heuchelin, 1990). No correlation was found between leaflet orientation scores and 24 h whole plant transpiration totals (Table 2.10).

Correlations between leaflet orientation and single plant yield were always positive but were never significant. This may indicate that the larger the leaflet orientation score, which in this study indicates low leaflet orientation, the higher the single plant yield, Correlations between leaflet orientation and replicated plot yields were very low (-0.12 to 0.08), not significant, and varied as to direction of correlation (Table 2.10). In 2005, analysis of the 210 population lines when separated into classes of leaflet orientation, detected significantly higher plot yields for lines which exhibited low leaflet orientation relative to high or medium leaflet orientation. Although the differences in the single plant yield analysis were not significant in the same data set, the same trend appeared in that lines with lower leaflet orientation were higher yielding than the high leaflet orienting class lines (Fig. 2.12). The two year data analysis of the 54 F4:6 and F7:8 population lines when separated into classes detected the same overall pattern in that the high leaflet orienting lines were always the lowest yielding in single plant yield relative to the other two classes. These differences were significant in the two year data analysis. However, no significant differences were detected between leaflet orientation classes for plot yield in the two year data analysis of the 54 F4:6 and F7:8 population lines. There were also no consistent patterns detected in the two individual years of the study as each class was observed to have the highest or lowest plot yield during that time period. However, the two year analysis did reflect the overall pattern of the single plant data in that low leaflet orienting lines were higher yielding than the high leaflet orienting lines (Table 2.9, Fig 2.13). These results conflict somewhat with observations by other researchers which indicate that by orienting upper canopy leaves, plants allow more light to penetrate into the lower canopy which may increase the overall amount of light interception and photosynthesis of the plant (Kawashima, 1969 a, b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000; Isoda and Wang, 2001). Such an increase in photosynthesis may be associated with increased yield (Wang et al., 1995; Chang and Taqumpay, 1970; Mickelson et al., 2002; Pendelton et al., 1968; Pepper et al., 1977). However it was also pointed out by Purcell (2006) that the main tenets of crop physiology are that crop mass and yield are proportional to both the cumulative amount of light intercepted and the amount of water transpired by the crop during a season. Indeed this study found a consistent and highly significant correlation ($r=0.62$, $p=<0.0001$) between whole plant transpiration and single plant yield in all data sets over the two year period. It is therefore possible that the trend of lower whole plant transpiration rates observed in the high leaflet orienting lines could have contributed to lower overall yields.

A significant correlation was detected between leaflet orientation and water use efficiency in the 2006 season of this study ($r = -0.28$, $p=0.04$). Although this association was not significant in the two year data analysis, it remained negative (Table 2.10). This may indicate that larger leaflet orientation score was weakly related to less water use per unit seed yield, as water use efficiency was defined in this study. No significant differences were found between the three leaflet orientation classes for water use efficiency over the two year period. However, the two year analysis reflected the same trend in that the low leaflet orienting lines used less water in producing the same unit of seed yield compared to the medium and high leaflet orienting lines (Table 2.9, Fig. 2.11). This finding is somewhat contradictory to other research that has associated high leaflet orientation to increased water use efficiencies (Pichard and Forseth, 1988; Rosa et al., 1991; Kao and Forseth, 1992; Bielenberg et al., 2003; Kao and Tsai, 1998). These previous studies defined water use efficiency in terms of gas exchange and carbon isotope discrimination whereas the current study defines it in terms water use during seed fill per unit of seed yield in a manner similar to Siahpoosh and Dehghanian, 2012. The results from the current study seems to indicate that in some cases, orienting leaflets may reduce leaflet temperatures, transpiration and photosynthetic rates to the point that efficiency of seed production may be decreased overall as it relates to water use and perhaps total yield.

Leaflet orientation scores were not associated with root morphology scores among the 210 F4:6 lines evaluated in 2005. Likewise in 2005, there was no significant correlation detected among the corresponding subset of 54 F4:6 lines which were chosen for further evaluation in the second year of the study. However a significant correlation was detected between leaflet orientation and root morphology scores in 2006 which resulted in an overall significant correlation ($r=0.33$, $p=0.02$) between the two traits over the two year period of the study (Table 2.10). Additionally, there were significant differences detected between the leaflet orientation classes for root morphology in both the 210 and 54 population line data sets over the two year period consistent with the correlations (Table 2.9, Figs. 2.10, 2.11). This would seem to indicate that plants with high leaflet orientation tended to have more normal tap root morphology and low leaflet oriented plant tended to have more prolific root morphology. These phenotypes of high orientation/normal root and low orientation/prolific root are consistent with that of the parental phenotypes and might indicate some degree of linkage. However, the difference in root scores between the high leaflet orienting class and the low leaflet orienting class was only 0.6 on a 1 to 5 scale, in the two year analysis (Table 2.9). This difference may be statistically significant but is likely not important as this small difference in visual rating would be nearly indistinguishable.

Significant correlations were detected between leaflet orientation and maturity in all data sets and across the two year period (r=0.34, p=0.01) (Table 2.10). Additionally, there were significant differences detected between the leaflet orientation classes for maturity in both the 210 F4:6 and 54 F7:8 population line data sets over the two year period consistent with the correlations (Table 2.11, Figs. 2.12, 2.13). Plants with larger leaflet orientation scores (low leaflet orientation) tended to be later in physiological maturity and high leaflet orientation lines tended to be earlier. These phenotypes of high orientation/earlier maturity and low orientation/later maturity are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of linkage.

Significant correlations were detected between leaflet orientation and seed size in all data sets and across the two year period (r=0.30, p=0.03) (Table 2.10). Additionally, there were significant differences detected between the leaflet orientation classes for seed size in both the 210 and 54 population line data sets over the two year period consistent with the correlations (Tables 2.8, 2.11). This would seem to indicate that plants with larger leaflet orientation scores (low leaflet orientation) tended to have larger seed and high leaflet orientation lines tended to have smaller seed. These phenotypes of high orientation/smaller seed and low orientation/larger seed are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of linkage.

Significant correlations were detected between leaflet orientation and seed oil in all data sets and across the two year period (r=-0.47, p=0.0003) (Table 2.10). Additionally, there were significant differences detected between the leaflet orientation classes for seed oil in both the 210 F4:6 and 54 F7:8 population line data sets over the two year period consistent with the correlations (Tables 2.8, 2.11). This indicates that plants with larger leaflet orientation scores (low leaflet orientation) tended to have lower seed oil content and high leaflet orientation lines tended to have higher seed oil content. These phenotypes of high orientation/higher seed oil and low orientation/lower seed oil are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of genetic linkage.

In the 2005 data set which contained 210 population lines, a significant correlation was detected between leaflet orientation and seed protein ($r=0.18$, $p=0.01$) (Table 2.10). Although the other data sets did not detect significant correlations, the association was always positive and similar in magnitude. Similarly only the 210 F4:6 population data set in 2005 detected significant differences between the leaflet orientation classes for seed protein, although this difference was very small (0.4%) (Table 2.8). Although no other data set analysis found significant differences between leaflet orientation classes and seed protein, the pattern of association between larger leaflet orientation score and higher seed protein content remained consistent (Table 2.11). This indicates that plants with low leaflet orientation tended to have higher seed protein content and high leaflet orientation lines tended to have lower seed protein content. These phenotypes of high orientation/lower seed protein and low orientation/higher seed protein are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of genetic linkage. This may also be related to the known negative correlation of seed protein and oil content in soybeans (Hymowitz et al., 1972; Wilcox and Shibbles, 2001; Chung et al., 2003).

Plants with lower leaflet orientation tended to be taller in plant height than higher leaflet orienting plants. This trend was only significant in the 2005 data set which contained the 210 population lines (Figure 2.12). The other data sets revealed the same pattern consistently but the differences were not significant (Table 2.11).

Plants with lower leaflet orientation tended to exhibit more lodging than higher leaflet orienting lines. This trend was significant in the 2005 data set which contained 210 F4:6 population lines (Table 2.8) and in the 2006 data set which contained 54 F7:8 population lines (Table 2.11). These differences were small (0.4 on a 1 to 5 scale) and although they may be statistically different, these differences are most likely not important as the visual rating differential would be nearly indistinguishable.

A significant correlation (r=0.42, p=0.002) was detected between leaflet orientation and leaf area in the 2006 data set analysis which contained 54 F7:8 population lines (Table 2.12). Additionally, there were significant differences detected between the leaflet orientation classes for leaf area (Table 2.13). This indicates that plants with low leaflet orientation tended to have larger leaf areas relative to higher leaflet orientation lines. These phenotypes of high orientation/lower leaf area and low orientation/higher leaf area are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of linkage. It has been reported previously that leaves which are shaded tend to be larger in size relative to leaves grown in full sunlight. It is an interesting speculation as to whether the overall increase in leaf area associated with the lower leaflet oriented lines could be, in some part, due to the shading effect of the upper canopy leaves relative to the lower canopy leaves. No data were collected in this study which would indicate this, however, a detailed study of upper, middle and lower canopy leaflet sizes could be beneficial in further explaining these results.

A significant correlation (r=0.42, p=0.002) was detected between lower leaflet orientation and dry weight biomass accumulation in the 2006 data set analysis which contained 54 F7:8 population lines (Table 2.12). Additionally, there were significant differences detected between the leaflet orientation phenotypic classes for dry weight biomass accumulation (Table 2.13). This indicated that plants with low leaflet orientation tended to have higher dry weight biomass accumulation and high leaflet orientation lines tended to have lower dry weight biomass accumulation. This is not surprising as higher seed yield, plant height, and leaf area were all found to be somewhat associated with the lower leaflet orienting lines in this study. Lower leaflet orienting lines were also somewhat associated with more prolific rooting which has been linked to higher biomass accumulation (Pantalone et al., 1999).

No significant correlations were detected between leaflet orientation scores and the traits of transpiration, stomatal conductance, and photosynthesis rates of upper canopy leaflet tissue exposed to full sunlight (Table 2.12). Additionally there were no significant differences detected between the three leaflet orientation classes for these traits (Table 2.13). This indicates that the lines within the three leaflet orientation classes were somewhat equal in their ability to transpire and photosynthesize at the leaflet level. Any differences therefore that might have been detected could have been more related to leaflet orientation differences than to differences in leaflet traits.

Root morphology effects

The frequency distribution of root morphology scores of lines developed and evaluated from this cross approximated a normal distribution, which suggests that the expression of this trait is polygenic in nature and is in agreement with observations by Pantalone et al., (1996b) (Fig. 2.14). The Shapiro-Wilk statistic for normality was (W=0.97, pr<W=0.0007) in the 2005 year analysis performed on the 210 F4:6 population lines which were selected somewhat randomly and without regard to the root morphology score. The phenotypic classes of normal, intermediate and prolific root morphology, formed by segregation of lines on the basis of their least squares mean root morphology scores separated significantly when analyzed as a phenotypic class for root morphology. This appears to validate that separation procedures were successful in creating distinct phenotypic classes (Tables 2.14, 2.15, Figs. 2.15, 2.16)

Significant correlations were found between root morphology scores and 24 h whole plant transpiration totals (r=0.19, p=0.005), (r=0.29, p=0.03) in the 2005 data sets which contained 210 and 54 F4:6 population lines, respectively. However, the 2006 correlation for the 54 F7:8 lines was slightly negative and not significant (r=-0.007, p=0.96). The two year correlation remained positive but was not significant (r=0.14, p=0.30) (Table 2.10). Thus in one of the two years, the prolific rooted plants tended to transpire more water over a 24 h period than plants with more normal tap roots. A significant difference was also detected between the normal root morphology class lines which tended to transpire less than the other two phenotypic classes in the 2005 set of 210 F4:6 population lines (Fig 2.15). Although the 2005, 2006 and two year data analysis of the phenotypic root classes containing the 54 F4:6 and F7:8 population lines found no significant differences in whole plant transpiration, the trend was consistent with that found in the correlation data (Table 2.15, Fig. 2.16). Previous studies have suggested that the unique prolific rooting morphology of PI 416937 may be a major component of its ability to tolerate drought (Hudak and Patterson, 1995, 1996; Chipman et al., 2001). Other studies have indicated that PI 416937 tolerates drought by means of limiting transpiration via osmotic regulation which decreases soil moisture loss throughout the growing period (Fletcher et al., 2007; Hudak and Patterson, 1996; King et al., 2009). The contribution of root morphology on this observed decrease in transpiration was previously unknown. In part I of this dissertation study the effect of rooting morphology on whole plant transpiration rates via grafting found no differences among root phenotype classes of prolific versus normal. The current study indicates that prolific rooting may allow plants to transpire at a higher rate relative to plants with a more normal tap root. The reason for this may be increased access to soil moisture due to the size, mass and soil area contact covered by the prolific root system, or perhaps water can more easily enter the prolific root phenotype for other reasons yet unknown.

Correlations between root morphology and single plant yield were always positive and were significant in the 2006 and two year data analyses ($r=0.29$, $p=0.03$), ($r=0.27$, $p=0.04$), respectively (Table 2.10). This indicates that the more prolific the root morphology the higher the single plant yield. Correlations between root morphology and replicated plot yields were very low ($r = -0.06$ to 0.09), not significant, and varied as to direction of correlation (Table 2.10). In 2005, analysis of the 210 F4:6 population lines when separated into classes of leaflet orientation detected significantly higher plot yields for lines which exhibited prolific rooting relative to normal tap rooted lines. Although the differences in the single plant yield analysis were not significant in the same data set, the same trend appeared in that lines with more prolific rooting were higher yielding than the normal tap rooted class lines (Fig. 2.17). The two year data analysis of the 54 F4:6 and F7:8 population lines when separated into classes detected the same overall pattern in that the prolific rooted lines were always higher in yield that the normal rooted lines. However, this difference was only significant in the 2006 analysis. No significant differences were detected between root morphology classes for plot yield in the two year data analysis of the 54 population lines. There were also no consistent patterns detected in the two individual years of the study as each class was observed to have the highest or lowest plot yield during that time period (Table 2.15, Fig 2.18). Previous studies have suggested a positive relationship between yield and increased root mass (Hammer et al., 2009; Lopes and Reynolds, 2010; Boyer et al., 1980; Jin et al., 2010). However, Pantalone et al. (1996b) found a non-significant but negative correlation ($r = -0.56$) between prolific rooting and yield. The current study generally supports previous findings that a relationship may exist between prolific rooting and productivity as related to increased single plant yield, although the interpretation of plot yield is not as distinguishable.

A significant correlation was detected between root morphology and water use efficiency in the 2006 season of this study ($r = -0.33$, $p = 0.01$). Although this association was not significant in the two year data analysis, it remained negative (Table 2.10). This seemed to indicate that more prolific rooting score was related to less water use per unit seed yield, as water use efficiency was defined in this study. However, no significant differences or patterns were found between the three root morphology classes and water use efficiency over the two year period (Table 2.15, Figs. 2.15, 2.16).

A significant correlation was detected between root morphology and maturity in the 2006 data analysis ($r=0.49$, $p=0.0002$) (Table 2.10). Although correlations for the other data sets were not significant, they were always positive indicating that prolific rooted plants in this population tended to be later in maturity. Additionally, there were significant differences detected between the root morphology classes for maturity in the 2005 data set which contained 210 F4:6 population lines (Fig. 2.17). The data analysis for the 54 F4:6 and F7:8 population line set over the two year period did not find significant differences between the root morphology classes, however the trend was consistent with the other analyses (Fig 2.18). These phenotypes of prolific rooting/later maturity and normal rooting/earlier maturity are consistent with that of the parental phenotypes and may indicate some degree of genetic linkage.

Significant negative correlations were detected between root morphology score and seed oil across the two year period (r=-0.28, p=0.04) (Table 2.10). Additionally, there were significant differences detected between the root morphology classes for seed oil in both the 210 F4:6 and 54 F7:8 population line data sets in 2005 and 2006, respectively (Tables 2.14, 2.16). This indicates that plants with more prolific rooting tended to have lower seed oil content relative to more normal tap rooted lines in this population. This is supports findings by Pantalone et al. (1996b) which also found negative correlations between prolific rooting scores and seed oil content. These phenotypes of prolific rooted/lower seed oil and normal rooted/higher seed oil are consistent with that of the parental phenotypes and may indicate some degree of genetic linkage.

No significant correlations were detected between root morphology scores and lodging, plant height, seed size or seed protein (Tables 2.14, 2.16, Figs. 2.17, 2.18). The 2005 and 2006 data sets which contained 210 F4:6 and 54 F7:8 population lines, respectively, detected differences in height such that the prolific rooted class appeared to be taller. However there was no significant difference in the other data sets over the two year period. Similarly the 2005 data sets detected differences in seed size such that the intermediate rooted class appeared to have larger seed. However there was no significant difference in the other data sets over the two year period. This is similar to Pantalone et al. (1996b) who found a positive but non-significant correlation between root morphology scores and seed size similar in magnitude to that found in the current study (r=0.12, p=0.36). The same study also found a significant positive correlation between prolific rooting and higher seed protein. Although this study failed to find any correlation or pattern of association between prolific rooting and higher seed protein, it did find an association between prolific rooting and lower seed oil. It may therefore be of importance to note that there was significant negative correlations detected (r=-0.38, p=0.003) in this study over the two year period between seed protein and oil similar in magnitude to what has been reported by Burton (1987) and Pantalone et al. (1996b).

Root morphology score and leaf area were significantly correlated (r=0.43, p=0.001) in the 2006 data set analysis which contained 54 F7:8 population lines (Table 2.12). Although there were no significant differences detected between the root morphology classes for leaf area, the same trend was evident (Table 2.17). This indicates that plants with more prolific rooting tended to have larger leaf areas relative to normal tap rooted lines. These phenotypes of prolific rooted/higher leaf area and normal rooted/lower leaf area are consistent with that of the parental phenotypes (Table 2.2) and may indicate some degree of genetic linkage.

A significant correlation (r=0.37, p=0.006) was detected between root morphology score and dry weight biomass accumulation in the 2006 data set analysis (Table 2.17).

Although there were no significant differences detected among the root morphology classes for dry weight biomass, the same trend was evident (Table 2.17). This indicates that plants with more prolific rooting tended to have higher biomass accumulation relative to normal tap rooted lines. This is in agreement with previous studies which have found positive relationships between root mass and biomass accumulation (Pantalone et al., 1999; Hammer et al., 2009; Jin et al., 2010).

No significant correlations were detected between root morphology scores and the traits of transpiration and stomatal conductance rates of upper canopy leaflets exposed to full sunlight (Table 2.12). Additionally there were no significant differences detected among the three root morphology classes for these traits (Table 2.17). This indicates that the lines within the three root morphology classes were somewhat equal in their ability to transpire at the leaflet level. Any differences therefore that might have been detected could have been more related to rooting morphology than to differences in leaflet traits.

A significant positive correlation ($r=0.49$, $p=0.0002$) was detected between root morphology score and photosynthesis rates of upper canopy leaflets exposed to full sunlight (Table 2.12). Additionally, the prolific rooting morphology class 2006 F7:8 population line analyses were significantly higher in photosynthetic rate than the other two classes (Table 2.17). This indicates that the F7:8 population lines in this study which expressed more prolific rooting tended to exhibit higher rates of photosynthesis in 2006. The exact reason for this is unknown although it is interesting that the PI 416937 parent which also expresses prolific rooting has also been characterized as having significantly higher rates of photosynthesis than the USG 5601T parent (Table 2.3).

Leaflet orientation and root morphology combination effects

The phenotypic combination classes of leaflet orientation and root morphology formed by segregation of lines on the basis of their least squares mean leaflet orientation and root morphology scores, separated significantly for each trait when analyzed as phenotypic classes. This appears to validate that separation procedures were successful in creating distinct phenotypic classes (Tables 2.18, 2.19, Figs. 2.19, 2.20)

The distribution of lines in the 2005 data set which contained 210 population lines, had larger numbers of medium leaflet oriented lines as might be expected from a large, somewhat randomly selected population. There also seemed to be slightly larger numbers of population lines which were classified as prolific rooted (86) than intermediate (65) or normal (59) (Table 2.18). This indicates that the population seems to skewed slightly towards prolific rooting. Additionally the prolific rooting seems to be associated in higher numbers with lower leaflet orientation as has been discussed previously.

No significant differences were detected between the combination classes which represent the extreme phenotypic combinations of High leaflet orientation/Normal root (H/N), Low leaflet orientation/Prolific root (L/P), High leaflet orientation/Prolific root (H/P), and Low leaflet orientation/Normal root (L/N) for whole plant transpiration, single plant yield, plot yield, or water use efficiency in any data set analyzed over the two year period (Tables 2.18, 2.19, Figs. 2.19, 2.20, 2.21, 2.22). Thus it appears that, in regards to these measured traits, there was no gain realized by combining the phenotypic attributes of leaflet orientation and root morphology. This is somewhat unexpected since correlations and phenotypic class analysis detected statistical differences for traits between the phenotypic extremes. This lack of an additive or reductive response could be due to the correlation between leaflet orientation and root morphology which was detected in this study. This would tend to confound the conclusion as to which of the phenotypic traits was responsible, in whole or in part, for the observed effect. Regarding whole plant transpiration and yield it is likely that the leaflet orientation is responsible for detected differences. In parts I and III of this dissertation study involving grafted plants and near-isogenic pairs, no effect was found on these traits when comparing normal roots to that of prolific roots. Additionally, it is the upper portion of the plant and its interaction with environmental aspects, such as the amount of PAR, which appears to be responsible for driving transpiration rates (Fig 2.9) and photosynthesis. Since water use efficiency in this study is calculated from the transpiration and associated single plant yields, it is therefore most likely that the leaflet orientation is responsible for detected effects.

Significant differences were detected among the combination classes which represent the extreme phenotypic combinations of H/N, L/P, H/P, and L/N for seed size. However, the pattern observed only reflected the previously described trends of larger seed being associated with lower leaflet orientation and no effect contributed by root morphology (Tables 2.18, 2.20).

Significant differences were also detected for maturity among the extreme phenotypic combination classes of H/N, L/P, H/P, and L/N. However, once again the pattern generally reflected the previously described trends of later maturity being associated with lower leaflet orientation and little to no effect contributed by root morphology (Tables 2.20, Figs. 2.21, 2.22).

Plant height differences were detected among the combination classes representing extreme phenotypic combinations of H/N, L/P, H/P, and L/N. Overall the trend observed was that of slightly increased plant height being associated with lower leaflet orientation and reflects the previously described pattern with little to no effect apparently contributed by root morphology (Tables 2.20, Figs. 2.21, 2.22).

Significant differences were detected for lodging among the extreme phenotypic combination classes of H/N, L/P, H/P, and L/N. However, the pattern largely reflected the previously described trend of slightly increased lodging being associated with lower leaflet orientation and little to no effect contributed by root morphology (Tables 2.20, Figs. 2.21, 2.22).

Seed oil differences were detected among the combination classes representing extreme phenotypic combinations of H/N, L/P, H/P, and L/N. The trend was lower seed oil being associated with lower leaflet orientation and reflects the previously described pattern with little to no effect being contributed by root morphology. There were no consistent significant differences detected between the extreme phenotypic classes for seed protein (Tables 2.20, Figs. 2.21, 2.22).

Significant differences detected among the combination classes which represent the extreme phenotypic combinations of H/N, L/P, H/P, and L/N for dry weight biomass accumulation. However, the pattern observed generally reflected the previously described trend of higher biomass accumulation being associated with lower leaflet orientation. The effect of the root morphology was somewhat uncertain as prolific rooting combinations were not consistently higher in biomass accumulation within class combinations of leaflet orientation (Table 2.21). There is a possibility that this result could be due to sample size since there was one class (H/P) which only contained three lines.

No significant differences were detected for leaf area among the extreme phenotypic combination classes of H/N, L/P, H/P, and L/N. Although the previously described pattern of lower leaflet morphology being associated with higher leaf area can be observed in the data, the effect of the root morphology is uncertain. The highest values of leaf area within each class of leaflet orientation were associated with normal, prolific and intermediate root morphology when combined with high, medium, and low leaflet orientation, respectively (Table 2.21). This may indicate that the leaflet orientation is more associated with the detected difference in leaf area in this population.

No significant differences were detected among the extreme phenotypic combination classes of H/N, L/P, H/P, and L/N for the traits of leaflet transpiration, stomatal conductance, and photosynthetic rates of upper canopy leaflet tissue exposed to full sunlight (Table 2.21). Thus it appears that, in regards to these measured traits, there was no gain realized when by combining the phenotypic attributes of leaflet orientation and root morphology. However, there was a pattern observed which generally reflected the previously described trend of higher photosynthesis rates being associated with prolific root morphology. This indicates that the lines within the extreme phenotypic combinations of H/N, L/P, H/P, and L/N were somewhat equal in their ability to transpire and photosynthesize at the leaflet level. Any differences therefore that might be detected could have been more related to leaflet orientation and rooting morphology than to differences in leaflet traits per se.

Chapter IV

Conclusions

The objective of this study was to investigate the effects of leaflet orientation and prolific rooting, both singly and in combination, on transpiration, seed yield, water use efficiency, leaflet temperature, leaflet photosynthesis rate, canopy light penetration, biomass production and other physiological and agronomic traits of soybean.

Parental evaluations

When comparing the upper and lower canopy leaflet levels of PAR, temperature, transpiration, stomatal conductance, and photosynthesis, both parental lines displayed decreases from upper to middle canopy. However, the high leaflet orienting parent line, USG 5601T, exhibited lower percentages of change from upper to middle canopy for all measured traits than the low leaflet orienting parent line, PI 416937.

Leaflet orientation effects

The frequency distribution of leaflet orientation scores of lines developed and evaluated from the cross of USG 5601T × PI 416937 approximated a normal distribution, which suggests that the expression of this trait is polygenic in nature. Average leaflet temperatures of random population lines over a two year period (2007-2008) which were exposed to partial sunlight as a result of paraheliotropism were an average of 5.2°C cooler than leaflets which did not orient their leaves and were exposed to full direct sunlight (Table 2.4, 2.5, Fig. 2.5).

Light penetration into middle canopy was significantly higher for lines which exhibited high leaflet orientation compared to lines that exhibited medium or low leaflet orientation (Table 2.7, Fig. 2.6).

No significant correlation was detected in this study between leaflet orientation and whole plant transpiration. A trend was noted in which the high leaflet orienting class transpired at a lower rate than the low or medium leaflet orienting classes, but that difference was not significant (Table 2.9, Fig. 2.11).

Consistently positive but non-significant correlations between leaflet orientation and single plant yield were observed in this study. Combined with the somewhat consistent and sometimes significant patterns of the replicated plot yield and single plant yield data of the leaflet orientation phenotypic class analysis, this indicated that low leaflet orientation appears to be associated with higher yield in this population. Purcell (2006) stated that the main tenets of crop physiology are that crop mass and yield are proportional to the cumulative amount of light intercepted and to the amount of water transpired by the crop during a season. This study found a consistent and highly significant correlation ($r=0.62$, $p=<0.0001$) between whole plant transpiration and single plant yield in all data sets over the two year period. It is therefore possible that the trend of lower whole plant transpiration rates observed in the high leaflet orienting lines may have contributed to lower overall yields.

Leaflet orientation and water use efficiency were negatively correlated in this study ($r = -0.28$, $p=0.04$). Combined with the somewhat consistent pattern found in the phenotypic class analysis, this indicated that low leaflet orientation was related to less water use per unit seed yield. The current study seems to indicate that in some cases, orienting leaflets reduces leaflet temperatures, transpiration and photosynthetic rates to the point that efficiency of seed production may be decreased overall as it relates to water use and perhaps total yield.

Leaflet orientation and root morphology scores were positively correlated($r=0.33$, $p=0.02$) among the population lines in this study over the two year period. This indicates that plants with high leaflet orientation tended to have more normal tap root morphology and low leaflet orienting plants tended to have more prolific root morphology. However, the difference in root scores between the high leaflet orienting and the low leaflet orienting phenotypic classes was only 0.6 on a one to five scale, in the two year analysis (Table 2.9). This difference may be statistically significant but is likely not important as this difference in visual rating would be nearly indistinguishable.

Significant correlations and phenotypic class analysis differences were detected between leaflet orientation and maturity ($r=0.34$, $p=0.01$), seed size ($r=0.30$, $p=0.03$), leaf area ($r=0.42$, $p=0.002$), seed oil ($r=-0.47$, $p=0.0003$) and protein content ($r=0.18$, $p=0.01$), in data sets across the two year period. This indicates that lines in this population with low leaflet orientation tended to exhibit later maturity, larger seed size, higher leaf areas, lower seed oil and higher seed protein content.

Significant correlation and phenotypic class analysis differences were detected between leaflet orientation and biomass accumulation ($r=0.42$, $p=0.002$) in this study. This indicates that lines with low leaflet orientation tended to exhibit higher biomass accumulation. Lower leaflet orienting lines were found to be somewhat associated with more prolific rooting in this study which has been linked to higher biomass accumulation previously (Pantalone et al., 1999). Additionally, lines in the low leaflet orientation phenotypic class tended to exhibit slightly higher plant height and lodging scores than higher leaflet orienting lines.

Root morphology effects

The frequency distribution of root morphology scores of lines developed and evaluated from this cross approximated a normal distribution, which suggests that the expression of this trait is polygenic in nature and is in agreement with observations by Pantalone et al. (1996b).

Significant correlations (r=0.19, p=0.005), (r=0.29, p=0.03) were found between root morphology scores and 24 h whole plant transpiration totals. This indicates that the more prolific rooted plants tended to transpire more water over a 24 h period during seed fill than plants with more normal tap roots. The current study indicates that prolific rooting may allow plants to transpire at a higher rate relative to plants with a more normal tap root. The reason for this may be increased access to soil moisture due to the size, mass and soil area contact covered by the prolific root system or perhaps water can more easily enter the prolific root phenotype for other reasons yet unknown.

Significant positive correlations (r=0.29, p=0.03), (r=0.27, p=0.04) and differences among phenotypic classes were detected between root morphology and single plant yield as well as some replicated plot yields. This indicates that prolific rooting was somewhat related to higher yield among the lines evaluated in this study. The current study generally supports previous findings that a relationship may exist between prolific rooting and productivity as related to increased single plant yield although the relationship with plot yield was not as distinguishable.

A significant negative correlation (r = -0.33, p = 0.01) was detected between root morphology and water use efficiency. Although this correlation was consistent, it was not always significant in all analyses. Furthermore, it was not supported by any differences or patterns in the phenotypic class analyses. It is therefore not clear if more prolific rooting was truly related to less water use per unit seed yield, as water use efficiency was defined in this study.

Significant correlations and phenotypic class analyses differences were detected in this study between root morphology and maturity (r=0.49, p=0.0002), seed oil (r=-0.28, p=0.04), and leaf area (r=0.43, p=0.001) indicating that prolific rooted plants tended to exhibit later maturity, lower seed oil content, and higher leaf areas relative to the normal tap rooted lines. No significant correlations were detected between root morphology scores and lodging, plant height, seed size or seed protein. However, some differences were detected among phenotypic root phenotypic class data sets for plant height which indicates prolific rooted plants tended to be taller.

Lines with more prolific rooting tended to have higher biomass accumulation relative to normal tap rooted lines (r=0.37, p=0.006). Lines evaluated in this study which expressed more prolific rooting tended to exhibit higher rates of photosynthesis (r=0.49, p=0.0002). The exact reason for this is unknown although it is interesting that the PI 416937 parent which also expresses prolific rooting has also been characterized as having significantly higher rates of photosynthesis than the USG 5601T parent.

Leaflet orientation and root morphology combination effects

No significant differences were detected among the extreme phenotypic combination classes of leaflet orientation and root morphology (H/N, L/P, H/P, and L/N) for whole plant transpiration, single plant yield, plot yield, water use efficiency, or seed protein content in any data set analyzed over the two year period. Thus it appears that, in regards to these measured traits, no gain was realized by combining the phenotypic attributes of leaflet orientation and root morphology

Significant differences were detected among the phenotypically extreme combination classes for seed size, maturity, plant height, lodging, seed oil content, dry weight biomass accumulation, and leaf area. However, the patterns observed only reflected the previously described trends of lower leaflet orientation being associated with larger seed size, later maturity, slightly increased plant height and lodging, lower seed oil, higher biomass accumulation, and higher leaf area. The root morphology phenotypes seemed to have little to no effect on the expression of these traits when analyzed as combined phenotypic classes.

Low leaflet orienting lines in this population were associated with prolific rooting, later maturity, larger seed size, higher leaf area, lower seed oil, and higher seed protein. Population lines exhibiting prolific rooting were associated with higher transpiration rates, later maturity, lower seed oil, higher leaf area, and higher upper canopy leaflet photosynthesis rates. High leaflet and normal rooted lines were associated with same traits but in the opposite manner. These phenotypic trait associations are consistent with the parental phenotypes suggesting some degree of genetic linkage.

Further study is needed to determine the effects of leaflet orientation and root morphology on whole plant transpiration, yield, water use efficiencies and other agronomic characteristics in soybeans. It is suggested that additional populations be created which allow the study of leaflet orientation and root morphology traits separately. Crosses between lines of the current population study could be crossed which would only segregate for leaflet orientation or root morphology. This would lessen the confounding effects that may have been encountered in the current study. Increased measurements of lines for leaflet traits of transpiration, stomatal conductance, and photosynthesis at different plant canopy levels are needed to better interpret the results. Additionally, recent improvements in the whole plant transpiration monitoring equipment greatly increases the capacity of plants measured simultaneously and should greatly reduce variation due to differing environmental conditions between days of measurement.

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APPENDIX

Part II

Table 2.1. Comparison of leaflet orientation, root morphology, whole plant transpiration, water use efficiency, single plant seed yield, and plot yield of parental lines (USG 5601T and PI 416937) evaluated in 2005 and 2006 in Tennessee.

Parental Line	Leaflet orientation class	Root morphology class	Leaflet orientation 2 yr	Root morphology 2 yr	Whole plant transpiration 2 yr	Water use efficiency 2 yr	Single plant seed yield 2 yr	Plot yield # 2 yr
			(score)†	(score)‡	(g H_20 $24h^{-1}$) §	(g H_2O $24h^{-1}$ g seed $yield^{-1}$)	(g $plant^{-1}$)	(kg ha^{-1})
USG 5601T	High	Normal	1.5 b ¶	1.3 b	389 b	18.2 b	22.5 b	3478 a
PI 416937	Low	Prolific	4.0 a	4.3 a	540 a	22.5 a	25.5 a	1777 b
Pr>F $_{.05}$			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on two or four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005; and 11 August and 15 September. 2006, respectively.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
# = plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 2.2. Comparison of leaflet orientation, root morphology, maturity, height, lodging, shattering, seed size, seed protein, seed oil, dry weight and leaf area of parental lines (USG 5601T and PI 416937) evaluated in 2005 and 2006 in Tennessee.

Parental Line	Leaflet orientation class	Root morphology class	Leaflet orientation 2 yr	Root morphology 2 yr	Maturity 2 yr	Plant height 2 yr	Lodging 2 yr	Shattering 2 yr	Seed size 2 yr	Seed protein 2 yr	Seed oil 2 yr	Dry weight 2006	Leaf area 2006
			(score)†	(score)‡	(DAP)	(cm)	(score)#	(score)††	(g 100 $seed^{-1}$)	(%)§	(%)§	(g $plant^{-1}$)	(cm^2 $plant^{-1}$)
USG 5601T	High	Normal	1.5 b ¶	1.3 b	140 b	88 a	1.5 b	1.1 b	15.1 b	41.6 b	20.4 a	65.5 a	2686 b
PI 416937	Low	Prolific	4.0 a	4.3 a	150 a	64 b	2.4 a	2.0 a	16.8 a	42.8 a	18.4 b	63.5 a	4440 a
Pr>F $_{.05}$			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
# = lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
†† = shattering = 1 to 5 scale; where 1 = no shattering seed loss; 2.5 = ~50% shattering and seed loss; 5 = 95+% shattering and seed loss.
DAP = days after planting.

Table 2.3. Percent reduction in rates of photosynthetically active radiation (PAR), leaflet temperature, leaflet transpiration, stomatal conductance, and photosynthetic rates between leaves of upper canopy and leaves of mid canopy of parental soybean lines USG 5601T and PI 416937 which differ in leaflet orientation.

Line	Leaflet orientation class	Position in canopy	Sunlight exposure	Leaflet orientation score	PAR ‡	Leaflet temperature ‡	Leaflet transpiration ‡	Stomatal conductance ‡	Photosynthesis ‡
				(score)†	(umol m^{-2} s^{-1})	Celsius	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(umol m^{-2} s^{-1})
USG 5601T	High Orientation	Upper	Full	1.5 b	1655 a §	35.7 b	7.1 b	0.63 b	17.9 b
USG 5601T	High Orientation	Middle	Partial Shade		430 b	35.3 b	6.1 b	0.40 b	7.3 c
				Percent Change:	-74.0%	-1.1%	-14.1%	-36.5%	-59.2%
PI 416937	Low Orientation	Upper	Full	4.3 a	1708 a	37.1 a	9.7 a	1.10 a	24.4 a
PI 416937	Low Orientation	Middle	Shaded		138 c	35.0 b	6.5 b	0.45 b	3.4 d
				Percent Change:	-91.9%	-5.7%	-32.9%	-59.1%	-86.1%
			$Pr>F_{.05}$	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = Measurements made with a Dynamax LCi photosynthesis meter on 6 and 8 September, 2006 between the hours of 1311 and 1523 and the hours of 1254 and 1341, respectively; each line / leaflet position treatment was measured four times on each day for a total of eight observations per treatment.

§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.4. Temperature differences of random soybean leaves with differing levels of sun exposure from the soybean population USG5601T × PI 416937 measured over the two year period (2007-2008) at Knoxville, TN.

Sun Exposure	2007 † Average Temperature	2008 ‡ Average Temperature	2 year Average Temperature
	---------------	Celsius	---------------
Full Sun	38.4 a §	38.0 a	38.2 a
Partial Sun (oriented leaf)	33.2 b	32.7 b	33.0 b
Shaded	28.8 c	30.3 c	29.4 c
$Pr>F_{.05}$	< 0.0001	< 0.0001	< 0.0113

† = 2007 60 replications on 17 September between the hours of 1245 and 1530 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface.
‡ = 2008 20 replications on 20 September between the hours of 1420 and 1535 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.5. Temperature differences of random soybean leaves differing in canopy position and levels of sun exposure from the soybean population USG5601T × PI 416937 measured over the two year period (2007-2008) at Knoxville, TN.

Position in canopy	Sun Exposure	2007 † Average Temperature	2008 ‡ Average Temperature	2 year Average Temperature
		---------------	Celsius	---------------
Upper	Full Sun	39.1 a §	37.8 a	38.4 a
Mid	Full Sun	37.6 b	38.2 a	37.9 a
Upper	Partial	33.3 c	33.3 b	33.3 b
Mid	Partial	33.1 c	32.1 c	32.6 b
Upper	Shaded	31.0 d	30.8 d	30.9 bc
Mid	Shaded	26.5 e	29.8 d	28.1 c
	$Pr>F_{.05}$	< 0.0001	< 0.0001	< 0.0013

† = 2007 30 replications on 17 September between the hours of 1245 and 1530 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface.
‡ = 2008 10 replications on 20 September between the hours of 1420 and 1535 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.6. Average Photosynthetically Active Radiation (PAR) levels at mid-canopy of high, medium, and low leaflet orientation lines including parents from the soybean population USG 5601T × PI 416937 at Knoxville, TN in 2006.

Leaflet orientation class / position	Average leaflet orientation score	Number of lines	PAR ‡
	(score)†		(umol m^{-2} s^{-1})
Ambient / above canopy			1539 a
High leaflet orientation lines	1.8 c §	5	75 b
Medium leaflet orientation lines	2.8 b	5	43 c
Low leaflet orientation lines	4.1 a	5	34 c
Pr>F $_{.05}$	< 0.0001		< 0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = PAR measurements made with a Dynamax LCi photosynthesis meter on 16 September, 2006 between the hours of 1448 and 1515; each line was measured once for a total of five mid-canopy PAR measurements per leaflet orientation class.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.7. Average Photosynthetically Active Radiation (PAR) levels at mid-canopy of high, medium, and low leaflet orientation lines including parents from the soybean population USG 5601T × PI 416937 at Knoxville, TN in 2008.

Leaflet orientation class / position	Average leaflet orientation score	Number of lines	PAR ‡
	(score)†		(umol m^{-2} s^{-1})
Ambient / above canopy			1747 a
High leaflet orientation lines	1.7 c §	5	383 b
Medium leaflet orientation lines	2.8 b	4	257 c
Low leaflet orientation lines	3.9 a	5	101 d
Pr>F $_{.05}$	< 0.0001		< 0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = PAR measurements made with a Decagon Sunflec Ceptometer on 17 September, 2008 between the hours of 1245 and 1337; each line was measured three times for a total of 12 to 15 mid-canopy PAR measurements per leaflet orientation class.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.8. Comparison of seed size, lodging, seed protein and oil of 210 F4:6 population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2005 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation	Seed size	Lodging	Seed Protein	Seed Oil
		(score)†	(g 100 seed^{-1})	(score)‡	(%)§	(%)§
High	51	2.0 c ¶	13.7 b	1.6 b	43.2 b	19.5 a
Medium	100	2.7 b	14.8 a	1.9 a	43.3 b	19.3 a
Low	59	3.4 a	15.2 a	2.0 a	43.6 a	18.6 b
Pr>F $_{.05}$		<0.0001	<0.0001	0.0075	0.0432	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.9. Comparison of whole plant transpiration, single plant seed yield, water use efficiency, and plot yield of 54 F4:6 and F7:8 soybean population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2005 and 2006 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation			Root morphology			Whole plant transpiration			Water use efficiency			Single plant seed yield			Plot yield #		
		2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr
		(score)†			(score)‡			(g H_20 24h^{-1}) §			(g H_2O 24h^{-1} g seed yield^{-1})			(g plant^{-1})			(kg ha^{-1})		
High	17	1.9 c ¶	1.9 c	1.9 c	2.5 a	2.2 b	2.3 b	566 a	446 b	504 b	27.7 a	21.4 a	23.7 a	21.1 b	24.6 a	22.9 b	2413 a	2751 a	2570 a
Medium	17	2.8 b	2.5 b	2.7 b	2.6 a	2.4 b	2.5 ab	662 a	550 a	596 a	25.3 a	20.2 a	23.2 a	27.0 a	29.2 a	27.5 a	2363 a	2703 a	2588 a
Low	20	3.6 a	3.5 a	3.6 a	2.8 a	2.9 a	2.9 a	659 a	464 ab	559 ab	28.0 a	17.2 a	22.1 a	24.2 ab	29.2 a	27.1 a	2631 a	2589 a	2611 a
Pr>F $_{.05}$		<0.0001	<0.0001	<0.0001	0.6177	0.0075	0.1044	0.5275	0.1024	0.1189	0.6418	0.1839	0.6566	0.1069	0.1501	0.0574	0.1635	0.3608	0.9400

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
# = plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 2.10. Correlations between leaflet orientation, root morphology, whole plant transpiration and several measured traits of soybean lines evaluated over the two year period 2005-6 at Knoxville, TN.

2005 (210 Lines)			**2005 (54 of 210 lines)**		**2006 (54 lines evaluated)**		**2005-6 (54 lines evaluated)**	
Leaflet orientation score† with:								
Trait	**Corr**	**(Pr>F)**	**Corr**	**(Pr>F)**	**Corr**	**(Pr>F)**	**Corr**	**(Pr>F)**
Root score‡	0.0843	0.2239	0.1298	0.3494	0.4864	0.0002 ††	0.3290	0.0152 ††
Transpiration §	0.0131	0.8509	0.0524	0.6969	-0.0726	0.6021	0.0603	0.6648
S. plant yield	0.0616	0.3745	0.1173	0.3981	0.1997	0.1477	0.1806	0.1913
WUE ¶	-0.0109	0.8753	0.0002	0.9987	-0.2848	0.0369 ††	-0.1042	0.4533
Plot yield #	0.0849	0.2206	-0.1204	0.3859	-0.0075	0.9572	-0.0229	0.8692
Seed size	0.3304	<0.0001 ††	0.4412	0.0008 ††	0.1589	0.2510	0.3039	0.0255 ††
Maturity	0.2919	<0.0001 ††	0.2940	0.0310 ††	0.2855	0.0364 ††	0.3368	0.0128 ††
Seed protein	0.1768	0.0102 ††	0.2410	0.0792	0.1444	0.2975	0.2033	0.1403
Seed oil	-0.3689	<0.0001 ††	-0.4589	0.0005 ††	-0.3909	0.0035 ††	-0.4697	0.0003 ††
Root morphology score‡ with:								
Transpiration §	0.1909	0.0055 ††	0.2888	0.0342 ††	-0.0068	0.9611	0.1429	0.3028
S. plant yield	0.1274	0.0654	0.2418	0.0781	0.2940	0.0310 ††	0.2736	0.0453 ††
WUE ¶	0.0732	0.2909	0.1154	0.4059	-0.3312	0.0144 ††	-0.0323	0.8166
Plot yld	0.0857	0.2164	-0.0621	0.6555	0.0116	0.9340	0.0541	0.6977
Seed size	-0.0002	0.9973	0.0749	0.5905	0.0164	0.9062	0.1268	0.3609
Maturity	0.1179	0.0884	0.0129	0.9260	0.4885	0.0002 ††	0.2512	0.0670
Seed protein	0.0276	0.6909	0.0739	0.5953	-0.0396	0.7764	-0.0026	0.9854
Seed oil	-0.1614	0.0192 ††	-0.0939	0.4995	-0.4905	0.0002 ††	-0.2875	0.0351 ††
Whole plant transpiration § with:								
S. plant yield	0.5001	<0.0001 ††	0.6285	<0.0001 ††	0.4978	0.0001 ††	0.6217	<0.0001 ††

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = whole plant transpiration measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.
¶ = WUE = water use efficiency = grams of water transpired in a 24h period per gram of single plant seed yield.
# = plot yield at the Knoxville location where other correlation traits were measured.
†† = correlation is significant α = 0.05 level.

Table 2.11. Comparison of maturity, height, lodging, seed size, seed protein and oil of 54 soybean population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2005 and 2006 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation 2005	2006	2 yr	Maturity 2005	2006	2 yr	Plant height 2005	2006	2 yr	Lodging 2005	2006	2 yr	Seed size 2005	2006	2 yr	Seed protein 2005	2006	2 yr	Seed oil 2005	2006	2 yr
			(score)†			(DAP)			(cm)			(score)‡			(g 100 $seed^{-1}$)			-------------------- (%)§ --------------------				
High	17	1.9 c ¶	1.9 c	1.9 c	138 b	146 b	142 b	69 a	68 a	69 a	1.6 a	2.2 b	1.9 a	13.0 b	15.5 b	14.3 b	43.3 a	41.9 a	42.6 a	19.4 a	19.1 a	19.3 a
Medium	17	2.8 b	2.5 b	2.7 b	142 b	145 b	143 b	71 a	68 a	69 a	1.6 a	2.6 ab	2.1 a	15.4 a	17.3 a	16.3 a	43.3 a	42.1 a	42.7 a	19.2 a	18.9 a	19.0 a
Low	20	3.6 a	3.5 a	3.6 a	153 a	158 a	155 a	75 a	73 a	74 a	1.6 a	2.6 a	2.1 a	15.6 a	16.6 ab	16.0 a	43.9 a	42.5 a	43.2 a	18.4 b	18.3 b	18.4 b
$Pr>F_{.05}$		<0.0001	<0.0001	<0.0001	0.0070	0.0354	0.0155	0.1716	0.2678	0.1621	0.9734	0.0788	0.4380	0.0014	0.0579	0.0099	0.1457	0.3521	0.2641	0.0002	0.0063	0.0007

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
DAP = days after planting.

Table 2.12. Correlations of leaflet orientation, root morphology, and whole plant transpiration, with leaf area, dry weight, transpiration, stomatal conductance and photosynthesis rates of 54 F7:8 soybean lines evaluated in 2006 at Knoxville, TN.

Leaflet orientation score† with:		
Trait	**Corr**	**(Pr>F)**
Leaf area	0.4187	0.0016 ¶
Dry weight	0.4208	0.0015 ¶
Leaflet transpiration §	-0.0585	0.6742
Stomatal conductance (Gs) §	-0.1219	0.3798
Photosynthesis §	0.1356	0.3283
Root morphology score‡ with:		
Leaf area	0.4313	0.0011 ¶
Dry weight	0.3723	0.0056 ¶
Leaflet transpiration §	0.1337	0.3351
Stomatal conductance (Gs) §	0.0877	0.5281
Photosynthesis §	0.4856	0.0002 ¶

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = leaflet transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight using the Dynamax LCi Photosynthesis meter on 8 September, 11 September, and 14 September between the hours of 1345 – 1707, 1355 – 1508, and 1226 – 1353, respectively.
¶ = correlation is significant α = 0.05 level.

Table 2.13. Comparison of dry weight, leaf area, leaf transpiration, stomatal conductance, and photosynthesis of 54 F7:8 soybean population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2006 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation	Dry weight	Leaf area	Full Sun Leaf Transpiration ‡	Full Sun Leaf Stomatal Conductance ‡	Full Sun Leaf Photosynthesis ‡
		(score)†	(g plant^{-1})	(cm^{2} plant^{-1})	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(umol m^{-2} s^{-1})
High	17	1.9 c §	45.6 c	2887 b	10.3 a	0.93 a	19.0 a
Medium	17	2.5 b	55.8 b	3291 ab	10.8 a	0.80 a	18.9 a
Low	20	3.5 a	66.1 a	3824 a	10.4 a	0.87 a	20.6 a
Pr>F $_{.05}$		<0.0001	0.0005	0.0168	0.6523	0.5299	0.2729

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = leaflet transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight using the Dynamax LCi Photosynthesis meter on 8 September, 11 September, and 14 September between the hours of 1345 – 1707, 1355 – 1508, and 1226 – 1353, respectively.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.14. Comparison of seed size, lodging, seed protein and oil of 210 F4:6 population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2005 at Knoxville, TN.

Root Morphology Class	Number of lines	Root morphology	Seed size	Lodging	Seed Protein	Seed Oil
		(score)†	(g 100 seed^{-1})	(score)‡	(%)§	(%)§
Normal	65	1.6 c ¶	14.4 b	1.7 a	43.3 a	19.4 a
Intermediate	86	2.5 b	15.1 a	1.8 a	43.5 a	19.1 b
Prolific	59	3.6 a	14.4 b	1.9 a	43.3 a	19.0 b
Pr>F $_{.05}$		<0.0001	0.0648	0.3207	0.4272	0.0082

† = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.15. Comparison of whole plant transpiration, single plant seed yield, water use efficiency, and plot yield of 54 F4:6 and F7:8 soybean population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2005 and 2006 at Knoxville, TN.

Root Morphology Class	Number of lines	Leaflet orientation 2005	2006	2 yr	Root morphology 2005	2006	2 yr	Whole plant transpiration 2005	2006	2 yr	Water use efficiency 2005	2006	2 yr	Single plant seed yield 2005	2006	2 yr	Plot yield # 2005	2006	2 yr
			(score)†			(score)‡			(g H_20 $24h^{-1}$) §			(g H_2O $24h^{-1}$ g seed $yield^{-1}$)			(g $plant^{-1}$)			(kg ha^{-1})	
Normal	18	2.5 b ¶	2.5 b	2.5 b	1.5 c	2.0 c	1.7 c	543 a	474 a	525 a	25.3 a	20.5 a	22.5 a	22.4 a	25.3 b	23.8 a	2418 a	2752 a	2637 a
Intermediate	20	2.9 ab	2.7 ab	2.8 ab	2.7 b	2.5 b	2.6 b	640 a	482 a	552 a	25.5 a	20.7 a	23.4 a	25.2 a	27.6 ab	26.0 a	2539 a	2648 a	2587 a
Prolific	16	3.1 a	3.0 a	3.0 a	3.8 a	3.2 a	3.5 a	718 a	504 a	587 a	31.1 a	16.7 a	22.8 a	24.6 a	30.8 a	28.0 a	2477 a	2628 a	2553 a
Pr>F $_{.05}$		0.0918	0.1417	0.0969	<0.0001	<0.0001	<0.0001	0.1911	0.8514	0.3977	0.1082	0.1915	0.8917	0.5693	0.1344	0.1671	0.7394	0.5536	0.7789

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.
¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.
# = plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 2.16. Comparison of maturity, height, lodging, seed size, seed protein and oil of 54 F4:6 and F7:8 soybean population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2005 and 2006 at Knoxville, TN.

Root Morphology Class	Number of lines	Root morphology 2005	2006	2 yr	Maturity 2005	2006	2 yr	Plant height 2005	2006	2 yr	Lodging 2005	2006	2 yr	Seed size 2005	2006	2 yr	Seed protein 2005	2006	2 yr	Seed oil 2005	2006	2 yr
			(score)†			(DAP)			(cm)			(score)‡			(g 100 $seed^{-1}$)		-------------------- (%)§ --------------------					
Normal	18	1.5 c ¶	2.0 c	1.7 c	141 a	147 a	144 a	69 a	67 c	68 a	1.5 a	2.4 a	1.9 a	14.0 b	16.1 a	15.0 a	43.5 a	42.2 a	42.9 a	19.1 a	18.9 ab	19.0 a
Intermediate	20	2.7 b	2.5 b	2.6 b	143 a	148 a	146 a	74 a	70 ab	72 a	1.7 a	2.5 a	2.1 a	15.6 a	17.1 a	16.3 a	43.6 a	42.3 a	43.0 a	19.1 a	19.0 a	19.0 a
Prolific	16	3.8 a	3.2 a	3.5 a	151 a	156 a	153 a	73 a	73 a	73 a	1.5 a	2.5 a	2.0 a	14.4 ab	16.2 a	15.3 a	43.5 a	42.0 a	42.7 a	18.7 a	18.4 b	18.5 a
Pr>F $_{.05}$		<0.0001	<0.0001	<0.0001	0.1454	0.2705	0.1881	0.4151	0.1515	0.2769	0.3903	0.7361	0.6630	0.0924	0.2933	0.1365	0.9205	0.7636	0.8255	0.2023	0.0941	0.1287

† = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.
DAP = days after planting.

Table 2.17. Comparison of dry weight, leaf area, leaf transpiration, stomatal conductance, and photosynthesis of 54 F7:8 soybean population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2006 at Knoxville, TN.

Root Morphology Class	Number of lines	Root morphology	Dry weight	Leaf area	Full Sun Leaf Transpiration ‡	Full Sun Leaf Stomatal Conductance ‡	Full Sun Leaf Photosynthesis ‡
		(score)†	(g $plant^{-1}$)	(cm^2 $plant^{-1}$)	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(umol m^{-2} s^{-1})
Normal	18	2.0 c §	52.0 a	3111 a	10.5 a	0.88 a	18.6 b
Intermediate	20	2.5 b	56.3 a	3283 a	10.3 a	0.79 a	18.9 b
Prolific	16	3.2 a	61.7 a	3740 a	10.8 a	0.94 a	21.4 a
$Pr>F_{.05}$		<0.0001	0.2465	0.1844	0.4884	0.4356	0.0339

† = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
‡ = leaflet transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight using the Dynamax LCi Photosynthesis meter on 8 September, 11 September, and 14 September between the hours of 1345 – 1707, 1355 – 1508, and 1226 – 1353, respectively.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.18. Comparison of seed size, lodging, seed protein and oil of 210 F4:6 population lines separated into nine combination classes consisting of high, medium, and low leaflet orientation and normal, intermediate and prolific root morphology evaluated in 2005 at Knoxville, TN.

Leaflet Orientation Class	Root Morphology Class	Number of lines	Leaflet orientation	Root morphology	Seed size	Lodging	Seed Protein	Seed Oil
			(score)†	(score)‡	(g 100 $seed^{-1}$)	(score)§	(%)¶	(%)¶
High	Normal	18	1.9 c #	1.6 c	13.2 e	1.6 bc	43.0 c	19.5 a
High	Intermediate	19	2.1 c	2.6 b	14.1 cde	1.4 c	43.4 abc	19.6 a
High	Prolific	14	2.1 c	3.5 a	13.7 de	1.6 bc	43.2 abc	19.4 ab
Medium	Normal	27	2.7 b	1.7 c	14.8 abcd	1.8 abc	43.3 abc	19.6 a
Medium	Intermediate	31	2.7 b	2.5 b	15.6 a	2.0 ab	43.4 abc	19.1 ab
Medium	Prolific	42	2.6 b	3.6 a	14.2 bcde	1.8 abc	43.2 bc	19.2 ab
Low	Normal	14	3.3 a	1.5 c	15.3 ab	1.7 bc	43.7 ab	19.1 b
Low	Intermediate	15	3.4 a	2.6 b	15.4 a	1.9 ab	43.8 a	18.4 c
Low	Prolific	30	3.4 a	3.5 a	15.1 abc	2.2 a	43.5 abc	18.5 c
$Pr>F_{.05}$			<0.0001	<0.0001	<0.0001	0.0276	0.2527	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
¶ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
# = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 2.19. Comparison of whole plant transpiration, water use efficiency, single plant seed yield, and plot yield of 54 F4:6 and F7:8 soybean population lines separated in to combination classes of high, medium, and low leaflet orientation and normal, intermediate and prolific root morphology evaluated in 2005 and 2006 at Knoxville, TN.

Leaflet Orientation Class	Root Morphology Class	Number of lines	Leaflet orientation			Root morphology			Whole plant transpiration			Water use efficiency			Single plant seed yield			Plot yield #		
			2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr
				(score)†			(score)‡			($g\ H_20\ 24h^{-1}$) §			($g\ H_2O\ 24h^{-1}\ g$ seed yield^{-1})			($g\ plant^{-1}$)			($kg\ ha^{-1}$)	
High	Normal	8	1.8 d ¶	1.8 d	1.8 c	1.5 d	1.8 d	1.6 c	480.6 b	443.0 a	472.2 b	26.8 a	18.4 ab	21.6 a	19.3 c	25.6 b	22.8 bc	2510.7 ab	2770.1 a	2633.9 a
High	Intermediate	6	2.0 d	2.1 cd	2.1 c	3.1 ab	2.2 cd	2.6 b	614.7 b	422.1 a	509.6 b	27.4 a	25.9 a	26.2 a	22.4 bc	22.0 b	21.6 c	2394.0 abc	2746.7 a	2550.7 a
High	Prolific	3	2.0 d	2.0 cd	2.0 c	3.9 a	3.3 ab	3.6 a	696.2 ab	503.8 a	575.0 ab	30.8 a	20.4 ab	24.4 a	23.1 bc	27.2 ab	25.5 bc	2176.3 bc	2706.2 a	2427.4 a
Medium	Normal	5	2.8 c	2.4 bc	2.6 b	1.6 cd	2.0 d	1.8 c	572.1 b	544.8 a	570.6 ab	23.3 a	25.6 a	25.0 a	26.1 abc	23.1 b	23.7 bc	1963.7 c	2775.2 a	2493.9 a
Medium	Intermediate	7	3.0 bc	2.4 bc	2.7 b	2.5 bc	2.6 c	2.5 b	507.5 b	530.5 a	538.9 b	21.9 a	19.2 ab	22.2 a	23.3 bc	29.4 ab	26.0 bc	2538.1 ab	2577.4 a	2553.0 a
Medium	Prolific	5	2.6 c	2.8 b	2.7 b	3.8 a	2.7 bc	3.3 a	968.5 a	583.7 a	702.4 a	32.0 a	16.1 b	22.9 a	33.2 a	35.1 a	33.4 a	2550.5 ab	2809.1 a	2685.9 a
Low	Normal	5	3.4 ab	3.6 a	3.5 a	1.5 d	2.2 cd	1.9 c	611.8 b	453.0 a	563.2 ab	24.7 a	18.6 ab	21.6 a	23.8 bc	26.8 ab	25.8 bc	2798.6 a	2698.2 a	2750.1 a
Low	Intermediate	7	3.7 a	3.4 a	3.6 a	2.7 b	2.7 bc	2.7 b	795.2 ab	485.2 a	600.8 ab	27.3 a	17.9 ab	22.2 a	29.5 ab	30.7 ab	29.9 ab	2635.2 ab	2653.2 a	2645.2 a
Low	Prolific	8	3.8 a	3.5 a	3.6 a	3.7 a	3.5 a	3.6 a	570.1 b	453.5 a	519.5 b	30.7 a	15.6 b	22.2 a	19.7 c	29.4 ab	25.6 bc	2544.5 ab	2485.7 a	2516.2 a
$Pr>F_{.05}$			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0666	0.7125	0.1520	0.6153	0.1203	0.8709	0.0470	0.1854	0.0477	0.1461	0.7796	0.9345

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.

¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

= plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 2.20. Comparison of maturity, height, lodging, seed size, seed protein and oil of 54 F4:6 and F7:8 soybean population lines separated into combination classes of high, medium, and low leaflet orientation and normal, intermediate and prolific root morphology evaluated in 2005 and 2006 at Knoxville, TN.

Leaflet Orientation Class	Root Morphology Class	Number of lines	Leaflet orientation			Root morphology			Seed size			Maturity			Plant height			Lodging			Seed protein			Seed oil		
			2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr	2005	2006	2 yr
				(score)†			(score)‡			(g 100 seed^{-1})			(DAP)			(cm)			(score)§		----------------------- (%)¶ -----------------------					
High	Normal	8	1.8 d #	1.8 d	1.8 c	1.5 d	1.8 d	1.6 c	12.3 d	15.6 ab	14.1 bc	143 ab	153 ab	148 ab	71 abc	70 abc	71 abc	1.7 ab	2.5 ab	2.1 ab	43.1 a	41.7 a	42.4 a	19.3 ab	18.9 abc	19.1 ab
High	Intermediate	6	2.0 d	2.1 cd	2.1 c	3.1 ab	2.2 cd	2.6 b	13.7 bcd	16.0 ab	15.0 abc	130 b	134 bc	132 b	65 bc	63 bc	64 bc	1.4 ab	1.8 b	1.6 b	43.5 a	42.0 a	42.8 a	19.7 a	19.7 a	19.7 a
High	Prolific	3	2.0 d	2.0 cd	2.0 c	3.9 a	3.3 ab	3.6 a	13.1 cd	14.4 b	13.6 c	140 ab	151 abc	146 ab	70 abc	73 ab	71 abc	1.5 ab	2.3 ab	1.9 ab	43.6 a	42.4 a	43.0 a	19.2 ab	18.5 bc	18.9 abc
Medium	Normal	5	2.8 c	2.4 bc	2.6 b	1.6 cd	2.0 d	1.8 c	14.9 abcd	16.4 ab	15.5 abc	130 b	132 c	131 b	62 c	61 c	62 c	1.1 ab	2.3 ab	1.7 ab	43.9 a	43.0 a	43.4 a	19.3 ab	19.1 ab	19.2 ab
Medium	Intermediate	7	3.0 bc	2.4 bc	2.7 b	2.5 bc	2.6 c	2.5 b	16.8 a	18.1 a	17.3 a	145 ab	150 abc	147 ab	79 a	68 abc	73 ab	2.0 a	2.7 a	2.4 a	43.3 a	42.0 a	42.6 a	19.1 ab	19.0 abc	19.0 ab
Medium	Prolific	5	2.6 c	2.8 b	2.7 b	3.8 a	2.7 bc	3.3 a	14.0 bcd	16.9 ab	15.6 abc	150 a	151 abc	151 a	69 abc	74 ab	71 abc	1.4 b	2.7 a	2.0 ab	42.9 a	41.4 a	42.1 a	19.2 ab	18.8 abc	19.0 abc
Low	Normal	5	3.4 ab	3.6 a	3.5 a	1.5 d	2.2 cd	1.9 c	15.6 abc	16.5 ab	15.9 abc	149 a	153 ab	151 a	73 abc	66 abc	70 abc	1.6 ab	2.4 ab	2.0 ab	43.8 a	42.4 a	43.1 a	18.6 bc	18.6 bc	18.6 bc
Low	Intermediate	7	3.7 a	3.4 a	3.6 a	2.7 b	2.7 bc	2.7 b	16.1 ab	16.9 ab	16.4 ab	153 a	158 a	155 a	77 ab	77 a	77 a	1.7 ab	2.9 a	2.3 a	44.0 a	43.0 a	43.5 a	18.6 bc	18.4 bc	18.5 bc
Low	Prolific	8	3.8 a	3.5 a	3.6 a	3.7 a	3.5 a	3.6 a	15.0 abc	16.4 ab	15.7 abc	155 a	162 a	158 a	76 ab	73 ab	74 ab	1.5 ab	2.5 a	2.0 ab	43.8 a	42.3 a	43.0 a	18.2 c	18.1 c	18.1 c
Pr>F $_{.05}$			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0121	0.3387	0.0962	0.0172	0.0442	0.0228	0.1284	0.1180	0.1354	0.4638	0.0815	0.3121	0.5193	0.4944	0.4976	0.0094	0.0356	0.0145

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
¶ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
# = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
DAP = days after planting.

Table 2.21. Comparison of dry weight, leaf area, leaf transpiration, stomatal conductance, and photosynthesis of 54 F7:8 soybean population lines separated into combination classes of high, medium, and low leaflet orientation and normal, intermediate, and prolific root morphology evaluated in 2006 at Knoxville, TN.

Leaflet Orientation Class	Root Morphology Class	Number of lines	Leaflet orientation (score)†	Root morphology (score)‡	Dry weight (g $plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Full Sun Leaf Transpiration § (mmol m^{-2} s^{-1})	Full Sun Leaf Stomatal Conductance § (mol m^{-2} s^{-1})	Full Sun Leaf Photosynthesis § (umol m^{-2} s^{-1})
High	Normal	8	1.8 d ¶	1.8 d	52.8 bc	3321.2 ab	10.2 a	0.99 ab	19.5 ab
High	Intermediate	6	2.1 cd	2.2 cd	36.2 d	2229.0 c	10.1 a	0.76 ab	16.6 b
High	Prolific	3	2.0 cd	3.3 ab	45.4 cd	3047.3 abc	11.2 a	1.15 a	22.1 a
Medium	Normal	5	2.4 bc	2.0 d	44.9 cd	2492.6 bc	10.1 a	0.67 b	16.2 b
Medium	Intermediate	7	2.4 bc	2.6 c	57.4 abc	3387.4 ab	10.9 a	0.83 ab	19.9 ab
Medium	Prolific	5	2.8 b	2.7 bc	64.5 ab	3953.8 a	11.2 a	0.88 ab	20.2 ab
Low	Normal	5	3.6 a	2.2 cd	57.7 abc	3393.5 ab	11.2 a	0.91 ab	19.3 ab
Low	Intermediate	7	3.4 a	2.7 bc	72.3 a	4082.8 a	9.9 a	0.79 ab	19.8 ab
Low	Prolific	8	3.5 a	3.5 a	66.0 ab	3866.1 a	10.4 a	0.91 ab	22.0 a
$Pr>F_{.05}$			<0.0001	<0.0001	0.0007	0.0087	0.5646	0.7171	0.0632

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
§ = leaflet transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight using the Dynamax LCi Photosynthesis meter on 8 September, 11 September, and 14 September between the hours of 1345 – 1707, 1355 – 1508, and 1226 – 1353, respectively.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

USG 5601 T PI 416937 1.5 score 4.5 score

Figure 2.1. Differences in leaflet orientation at different times of day. Leaflet orientation score is a phenotypic rating on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining an angle parallel to the horizontal plane.

USG 5601T PI 416937

Figure 2.2. Visual rating scale used in scoring root morphology. Root morphology score is a phenotypic rating on a scale of 1 to 5 with 1 being the condition of the plant possessing a normal tap root with few lateral roots and 5 being the condition of the plant possessing a prolific root mass with many fibrous-like lateral branching roots.

Figure 2.3. Use of Dynamax Flow32 System (fitting Dynagauges to soybean stem): a) each plant marked with durable tag for later identification, b) stem diameter measured and recorded, c) stem cleaned of dirt and debris, d) Dynagauge sensor placed around stem with top and bottom sealed with adhesive putty to prevent water and insect infiltration, e) insulating bubble wrap foil placed around Dynagauge (3 layers) and held in place with cable ties securely but with only light pressure, f) part of the Dynamax Flow 32 Sap Monitoring System setup as used in the field experiment showing the attachment to an upright cart for greater mobility, deep cycle marine battery and data link cable inside tool box at bottom, and portable computer for uploading program parameters and collecting data.

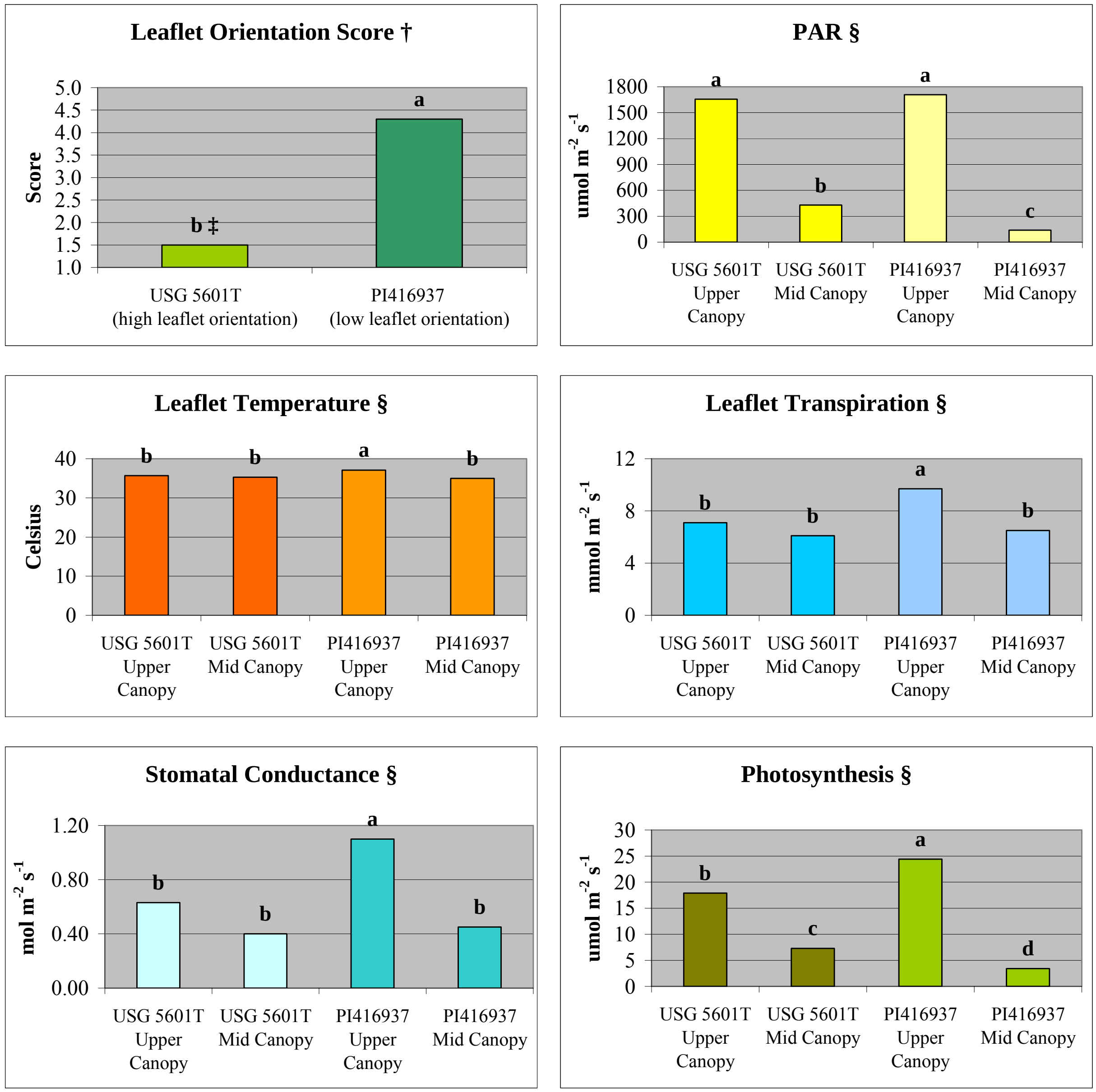

Figure 2.4. Leaflet orientation score, photosynthetically active radiation (PAR) level, leaflet temperature, leaflet transpiration, stomatal conductance, and photosynthetic rates of upper canopy and mid canopy leaves of soybean lines differing in leaflet orientation. USG 5601T exhibits high leaflet orientation; PI 416937 exhibits a low degree of leaflet orientation.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

§ = Measurements made with a Dynamax LCi photosynthesis meter on 6 and 8 September, 2006 between the hours of 1311 and 1523 and the hours of 1254 and 1341, respectively; each line / leaflet position treatment was measured four times on each day for a total of eight observations on per treatment.

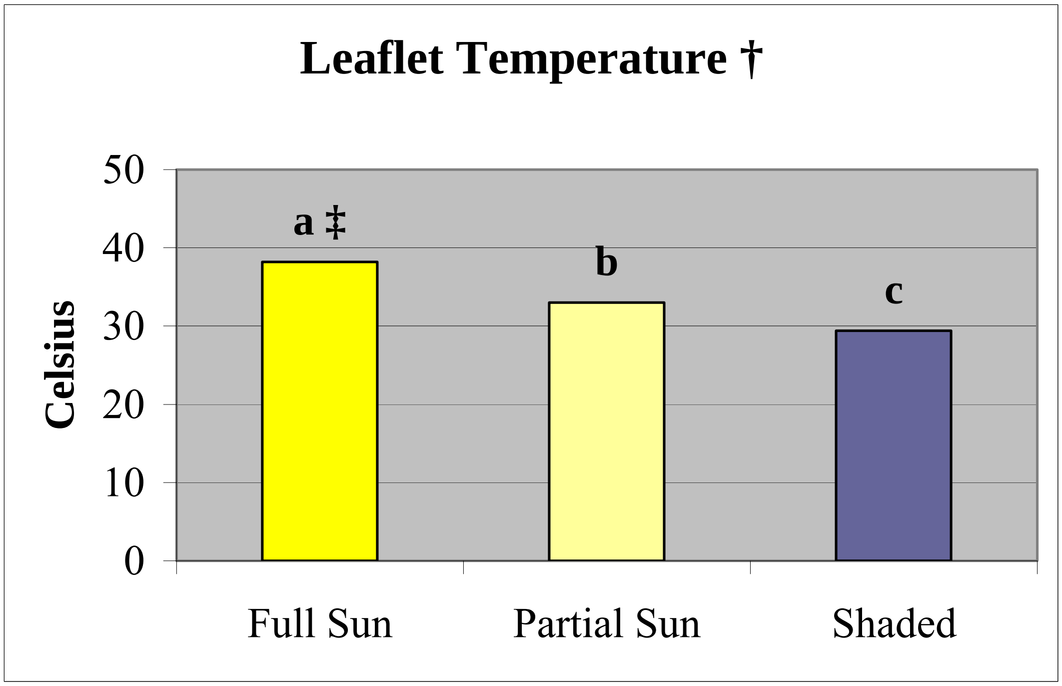

Figure 2.5. Temperatures of random soybean leaves with differing levels of sun exposure from the soybean population USG 5601T × PI 416937 averaged over a two year period (2007-2008).

† = 2007 60 replications on 17 September between the hours of 1245 and 1530 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface; 2008 20 replications on 20 September between the hours of 1420 and 1535 using a Raytek infrared thermometer at a distance of 4-6 inches from leaf surface.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

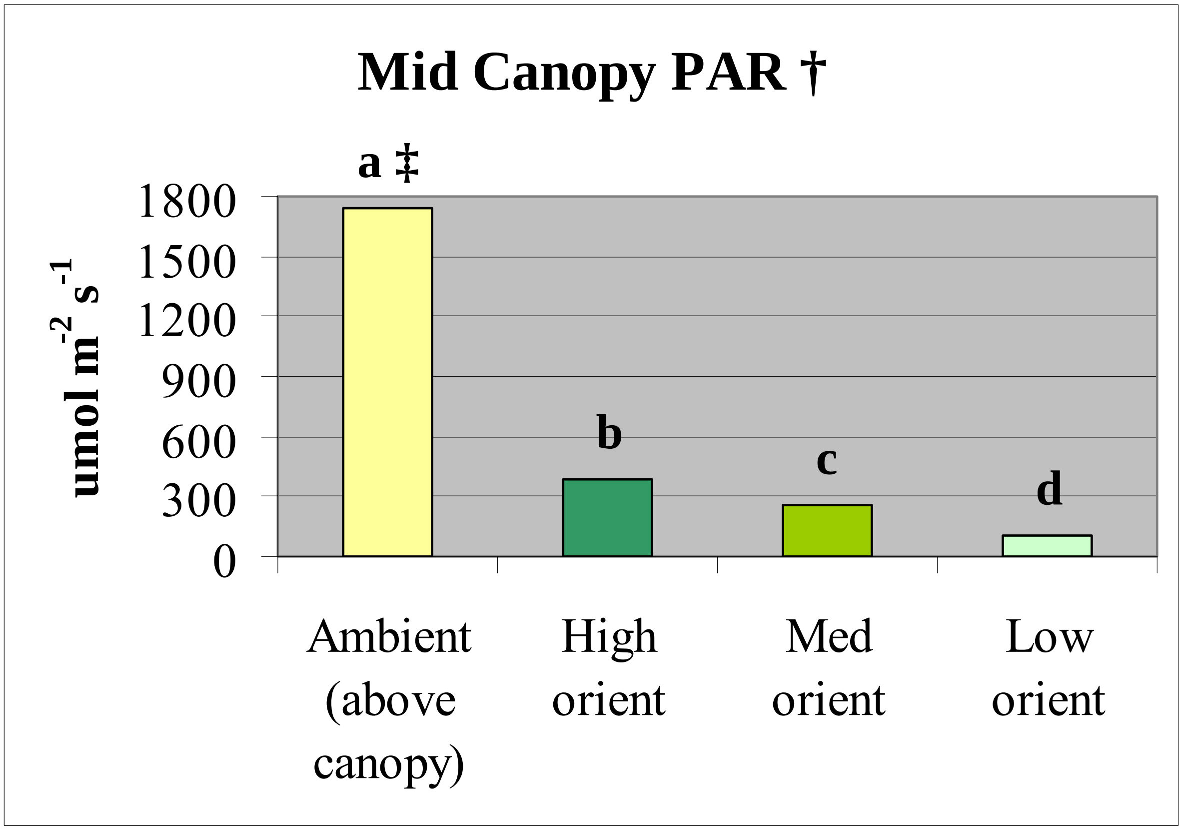

Figure 2.6. Photosynthetically Active Radiation (PAR) levels above and at mid-canopy of high, medium, and low leaflet orientation lines and parents from the soybean population USG 5601T × PI 416937 measured at Knoxville, TN in 2008.

† = PAR measurements made with a Decagon Sunflec Ceptometer on 17 September, 2008 between the hours of 1245 and 1337; each line was measured three times for a total of 12 to 15 mid-canopy PAR measurements per leaflet orientation class.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

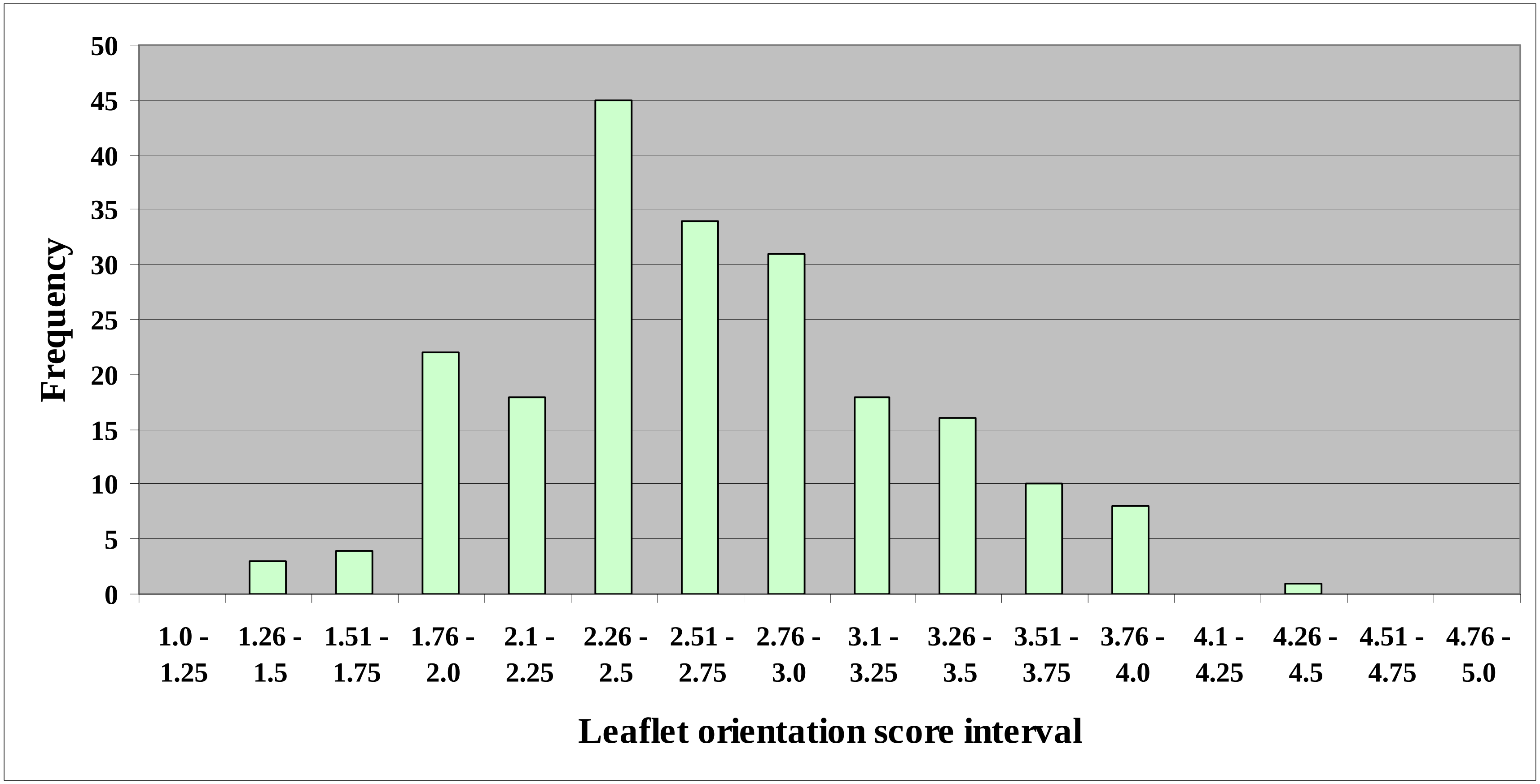

Figure 2.7. Frequency distribution of phenotypic leaflet orientation scores for 210 individual F4:6 soybean plants grown at Knoxville, TN in 2005.

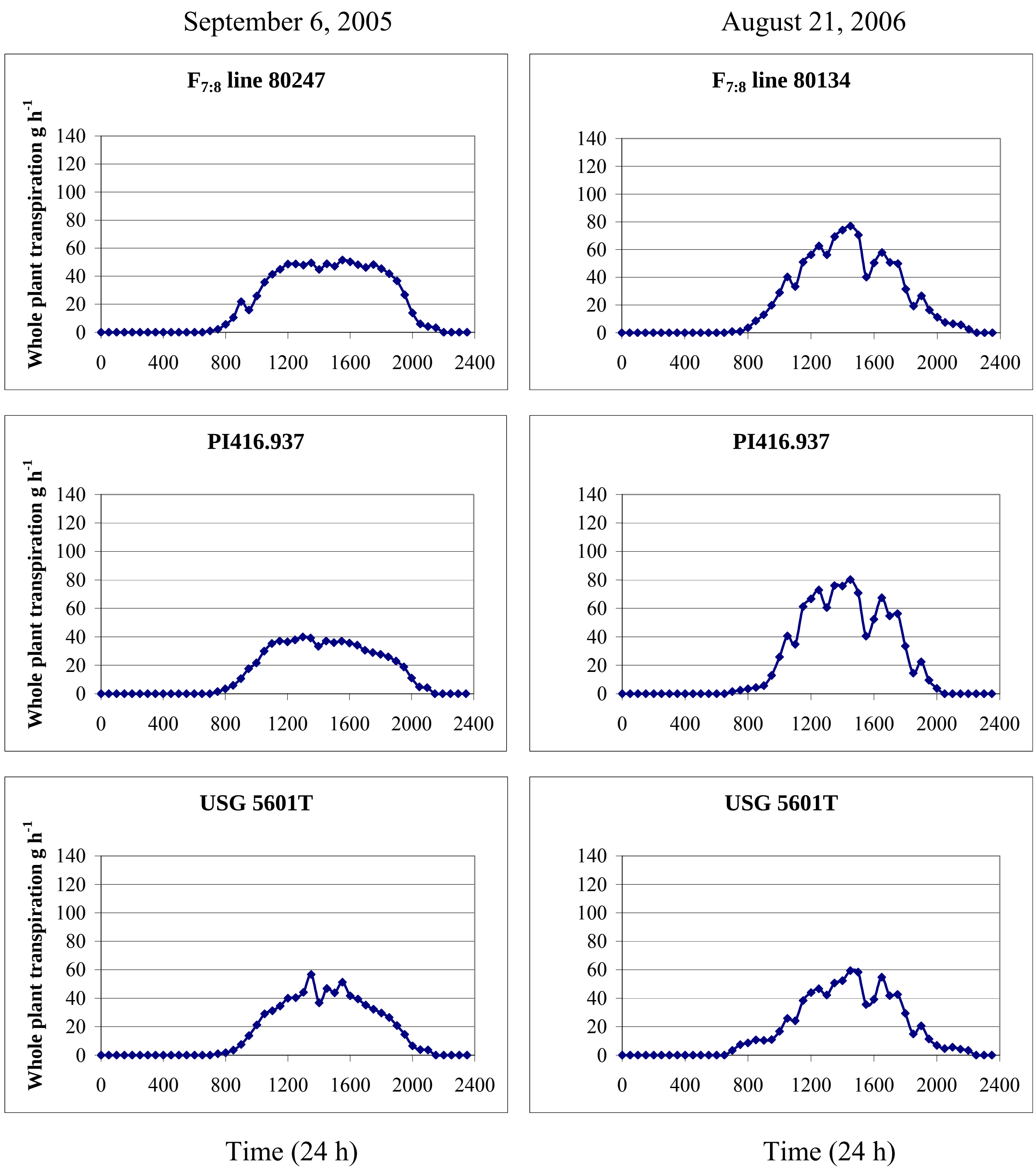

Figure 2.8. Whole plant transpiration measurements two different F7:8 population lines and parents recorded during two different 24 h periods over the two year period, 2005 and 2006. Although transpiration curves displayed are from different measurement days, each is representative of the total average flow for the line that year. The selected lines are represented in each of the measurement days noted in this figure in order to demonstrate similarities in the whole plant transpiration curves across different lines within a given day. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR leaflet temperatures, and transpiration.

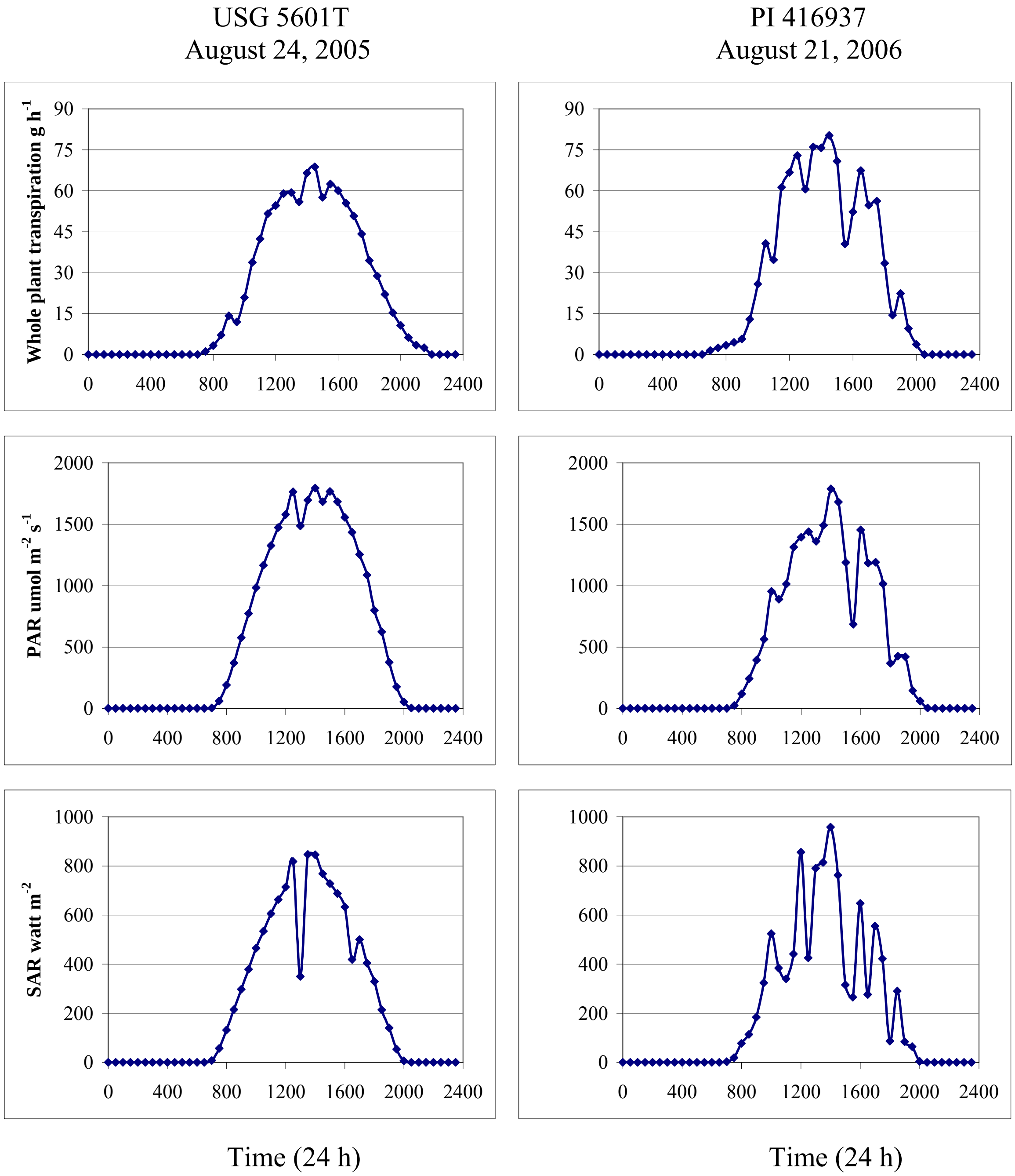

Figure 2.9. Whole plant transpiration, photosynthetically active radiation and solar radiation measurements of parental lines (USG 5601T, PI 416937) recorded during two different 24 h periods in 2005 and 2006. The similarity in the curves within each day demonstrates the close relationship between PAR and transpiration. The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration.

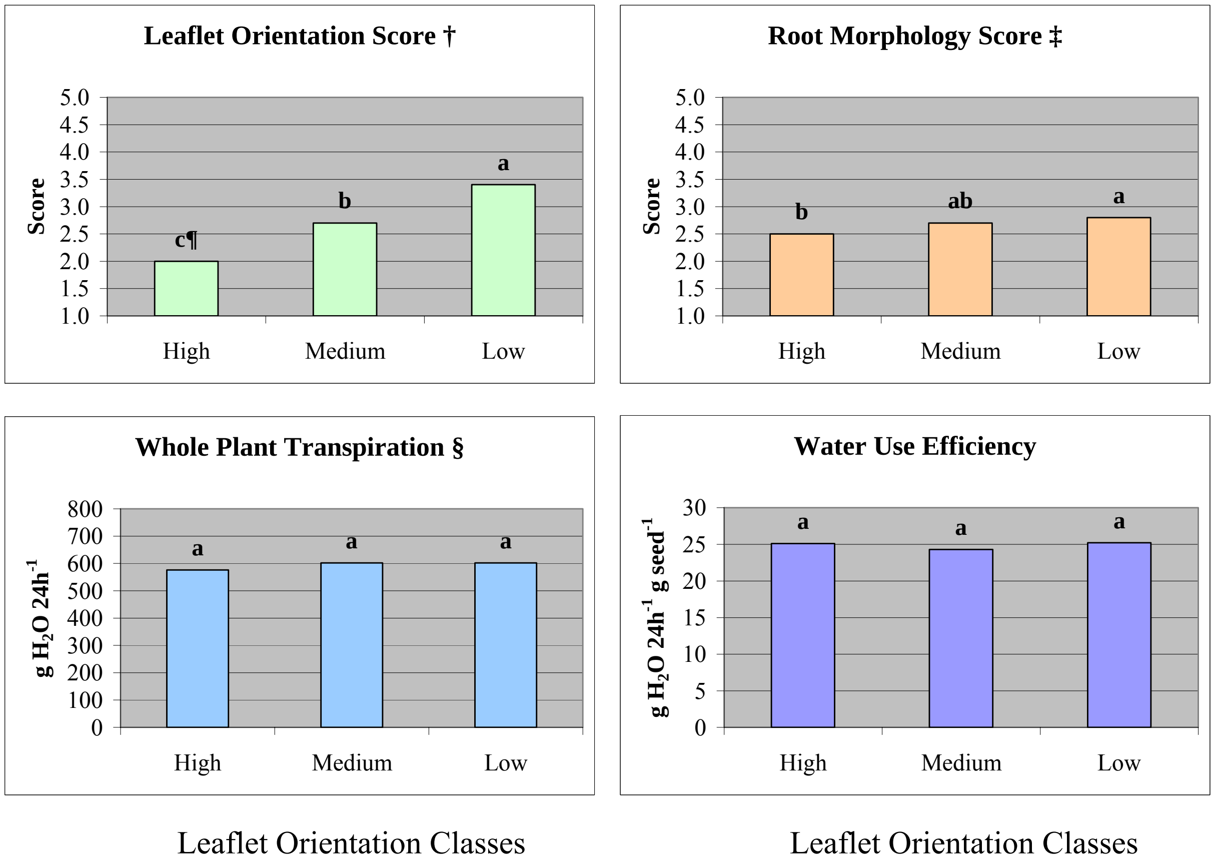

Figure 2.10. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 210 F4:6 population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2005 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on two plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

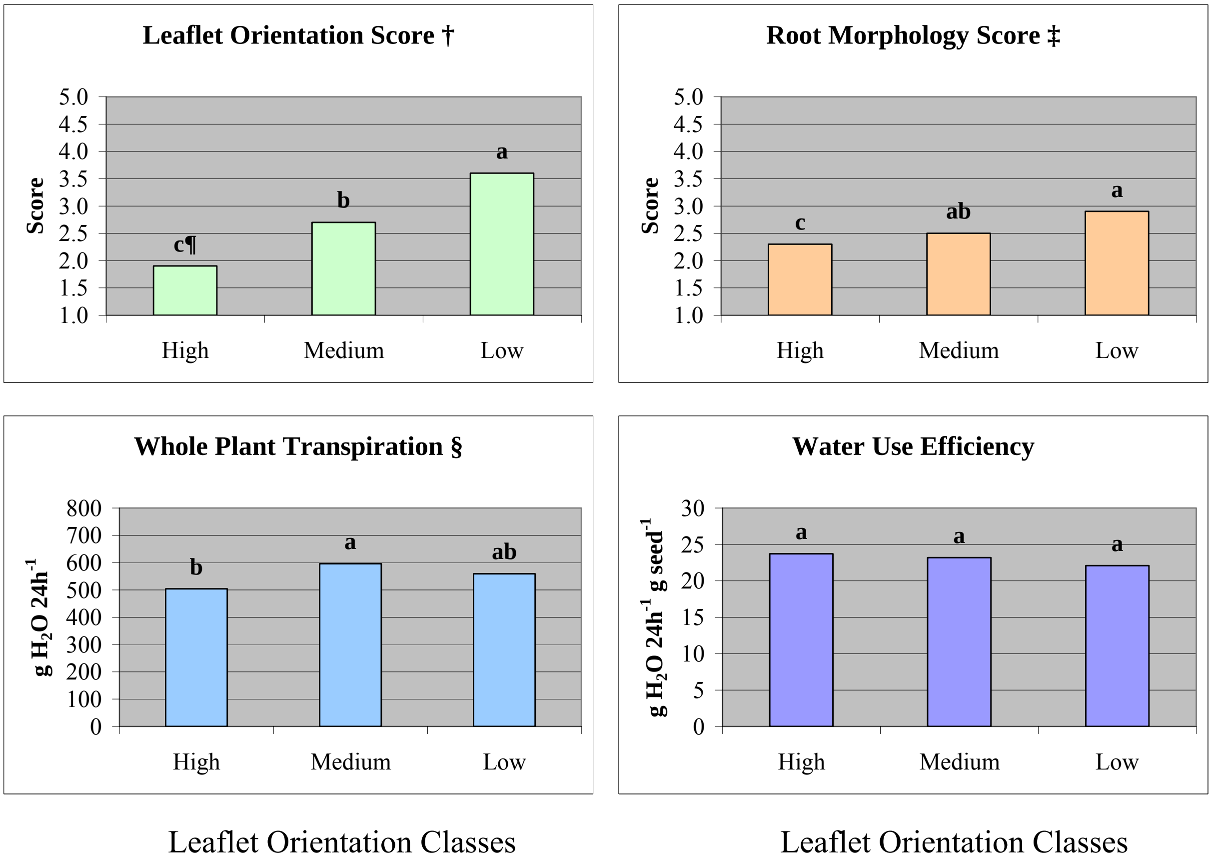

Figure 2.11. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 54 F4:6 and F7:8 population lines separated into classes of high, medium, and low leaflet orientation evaluated for two years, 2005-2006 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.

¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

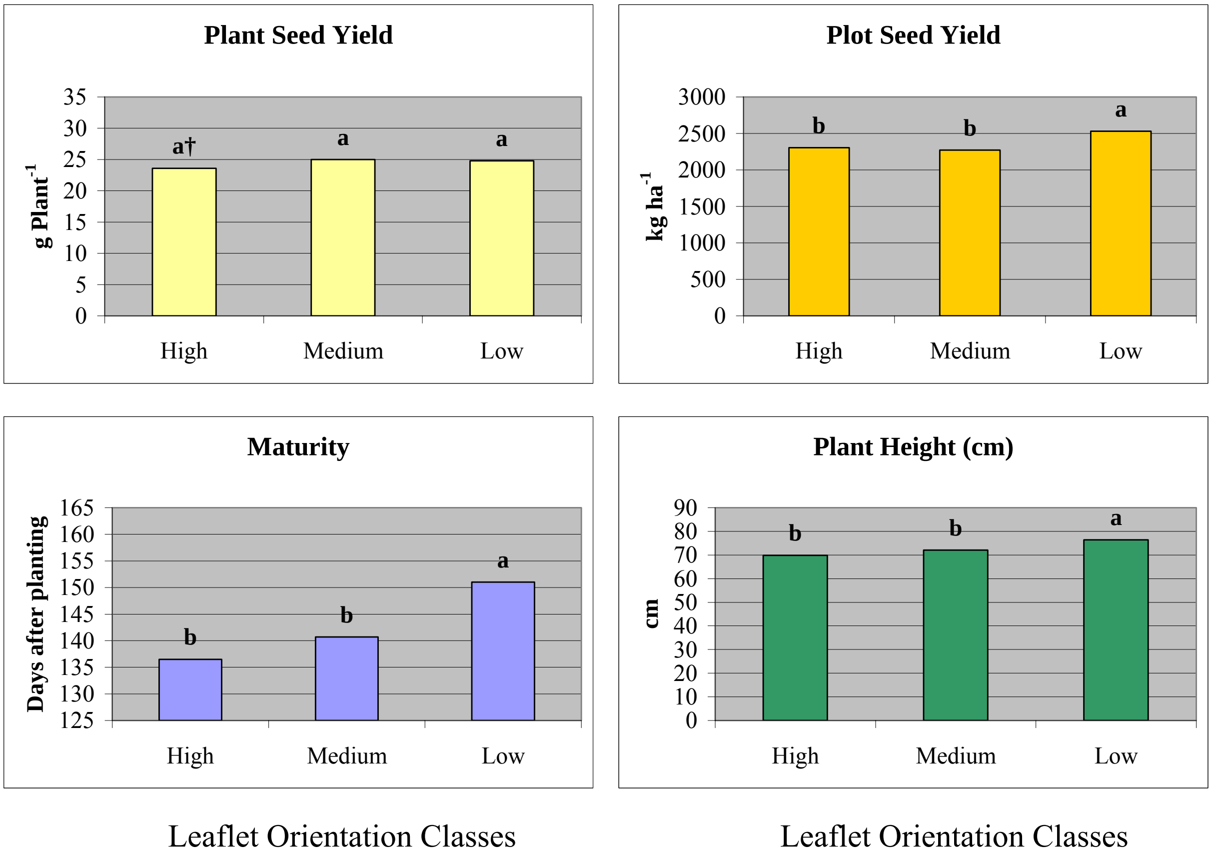

Figure 2.12. Comparison of plant seed yield, plot seed yield, maturity and plant height of 210 F4:6 population lines separated into classes of high, medium, and low leaflet orientation evaluated in 2005 at Knoxville, TN.
† = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

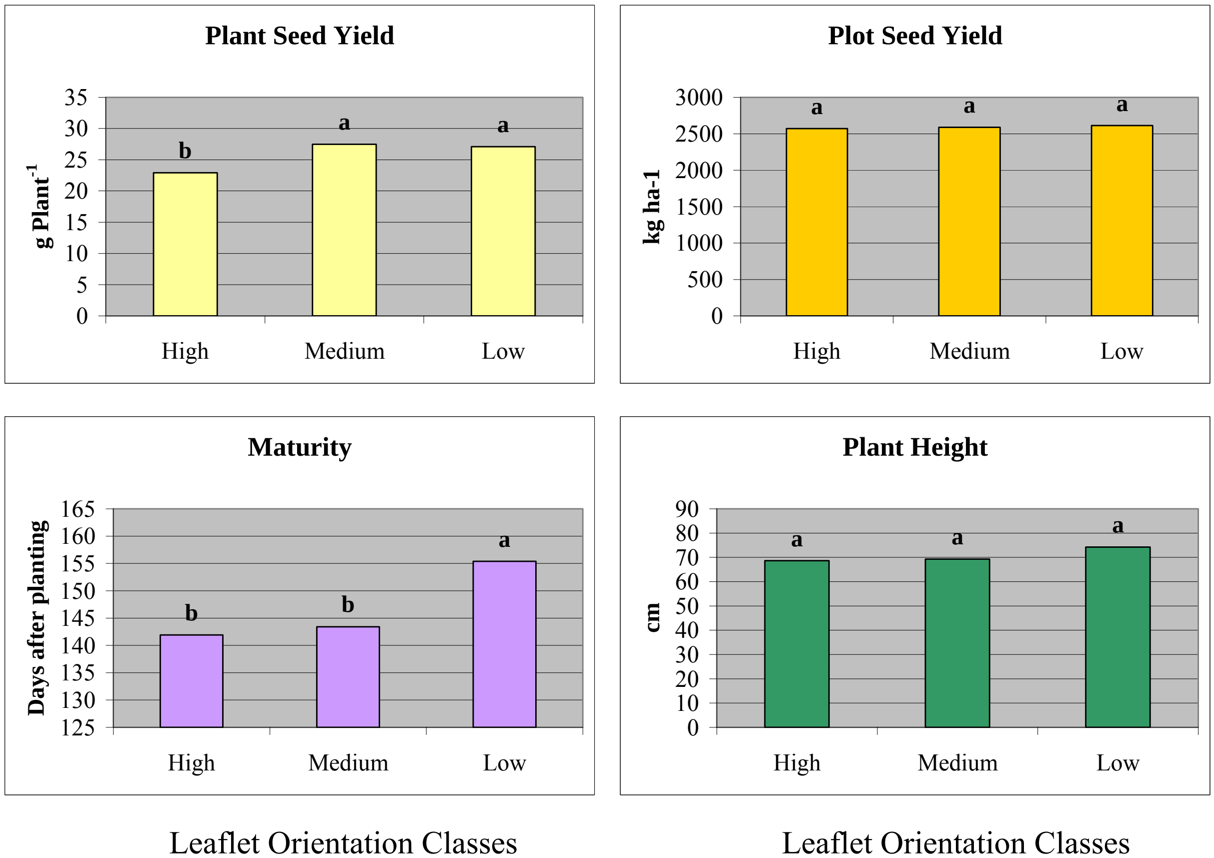

Figure 2.13. Comparison of plant seed yield, plot seed yield, maturity and plant height of 54 F4:6 and F7:8 population lines separated into classes of high, medium, and low leaflet orientation evaluated for two years, 2005-2006 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

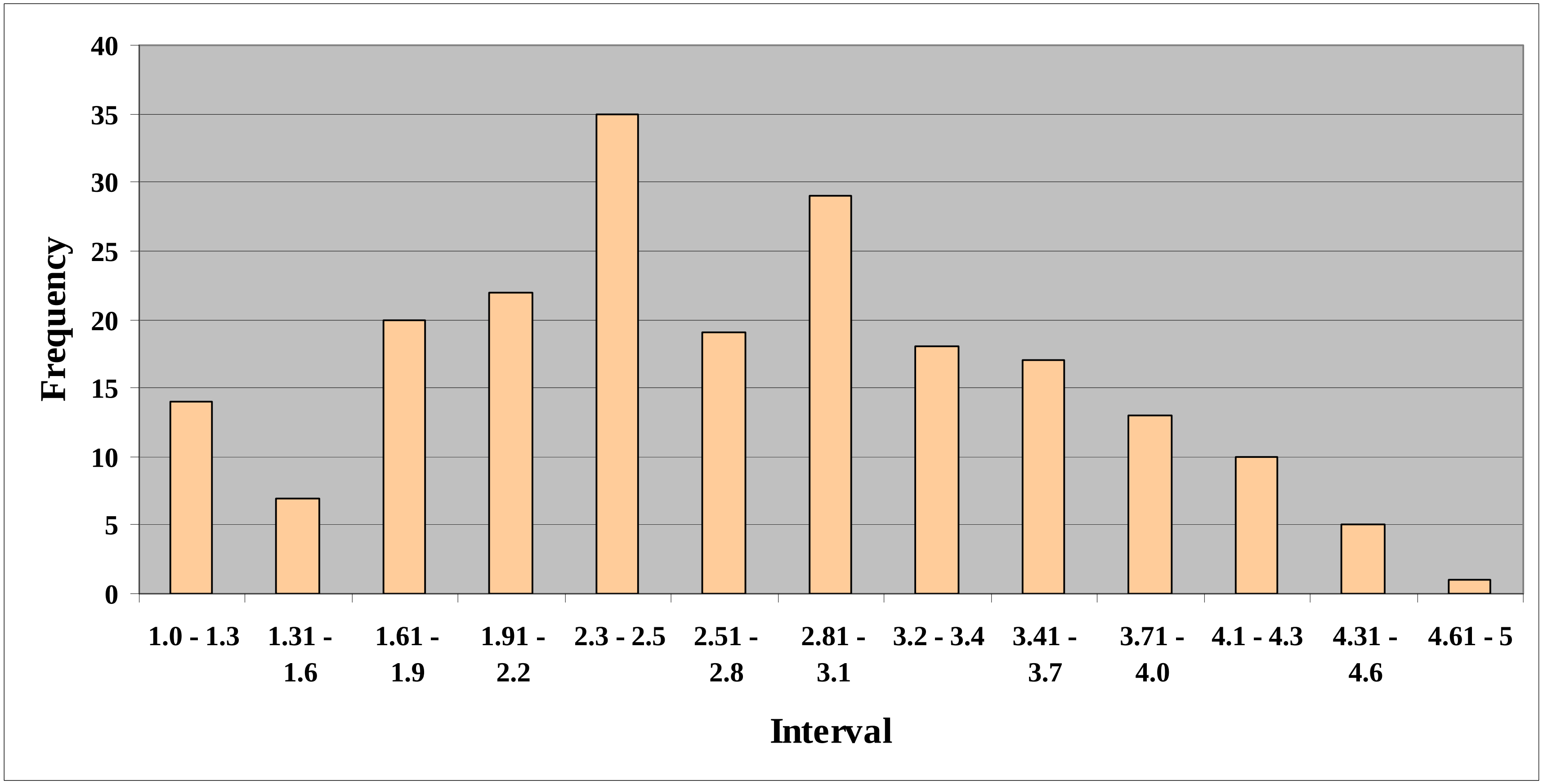

Figure 2.14. Frequency distribution of phenotypic root morphology scores for 210 individual F4:6 soybean plants grown at Knoxville, TN in 2005.

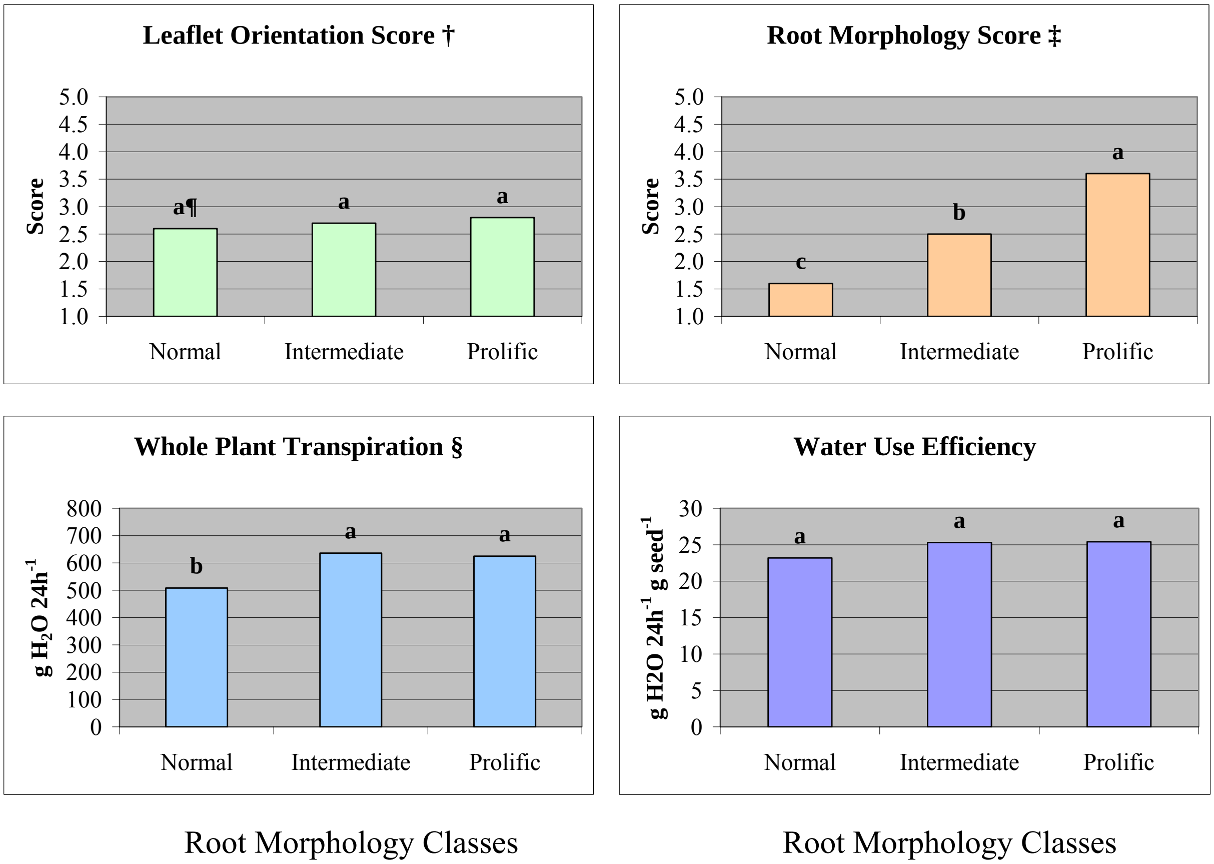

Figure 2.15. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 210 F4:6 population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2005 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.

¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

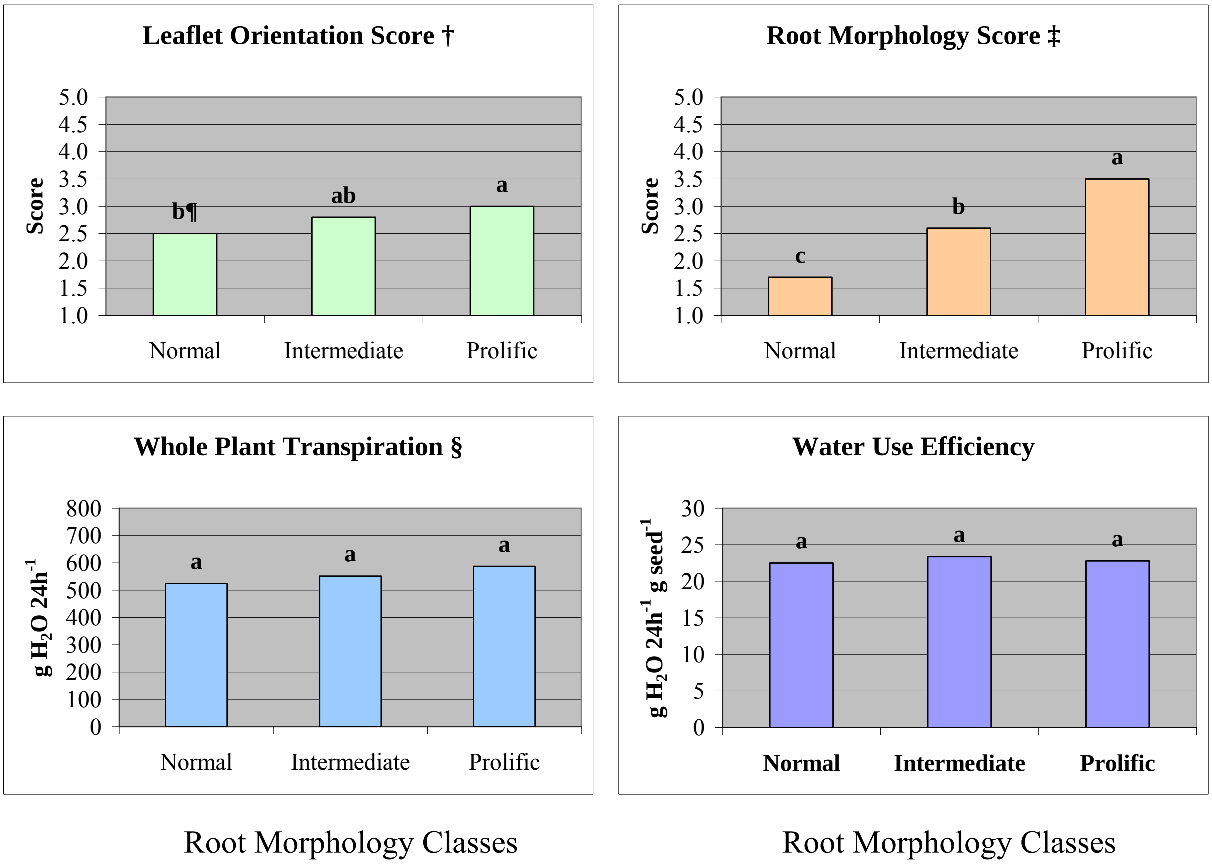

Figure 2.16. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 54 F4:6 and F7:8 population lines separated into classes of normal, intermediate, and prolific root morphology evaluated for two years, 2005-2006 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.

¶ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

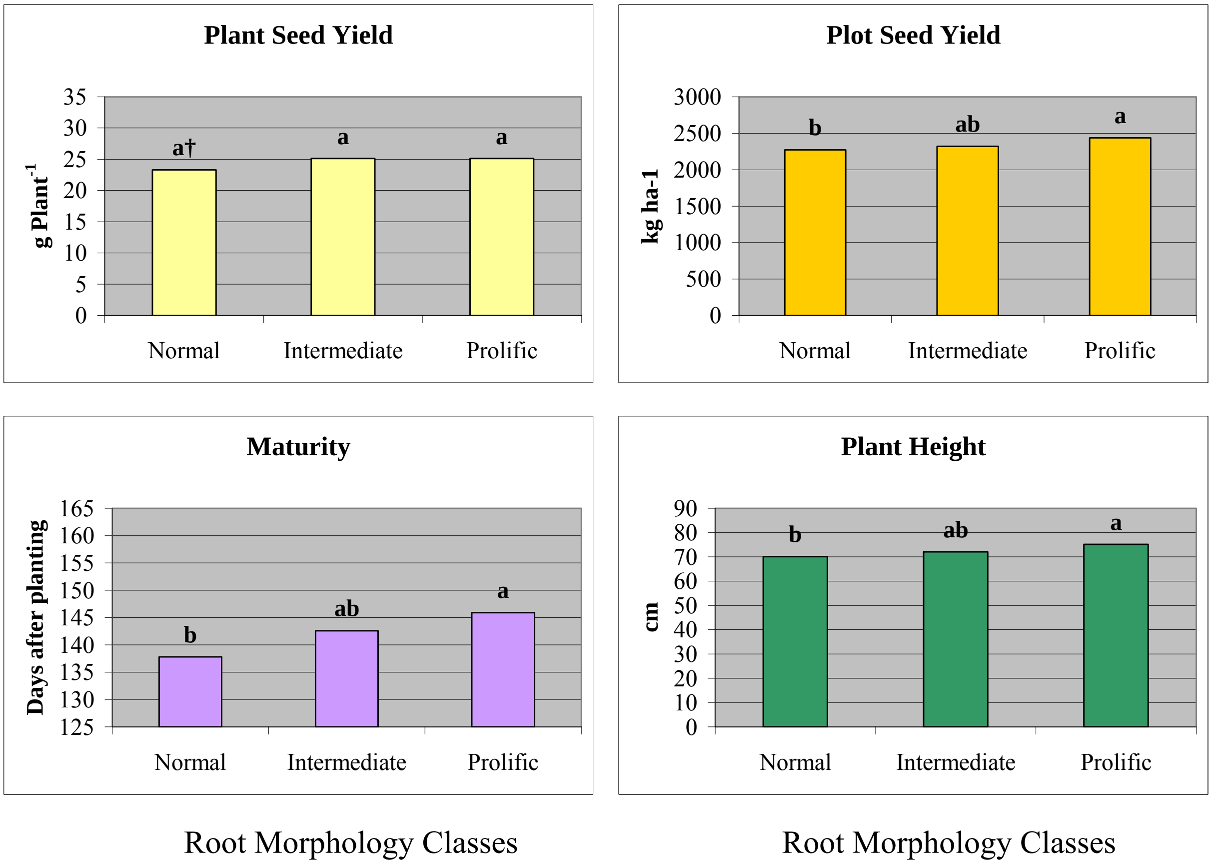

Figure 2.17. Comparison of plant seed yield, plot seed yield, maturity and plant height of 210 F4:6 population lines separated into classes of normal, intermediate, and prolific root morphology evaluated in 2005 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

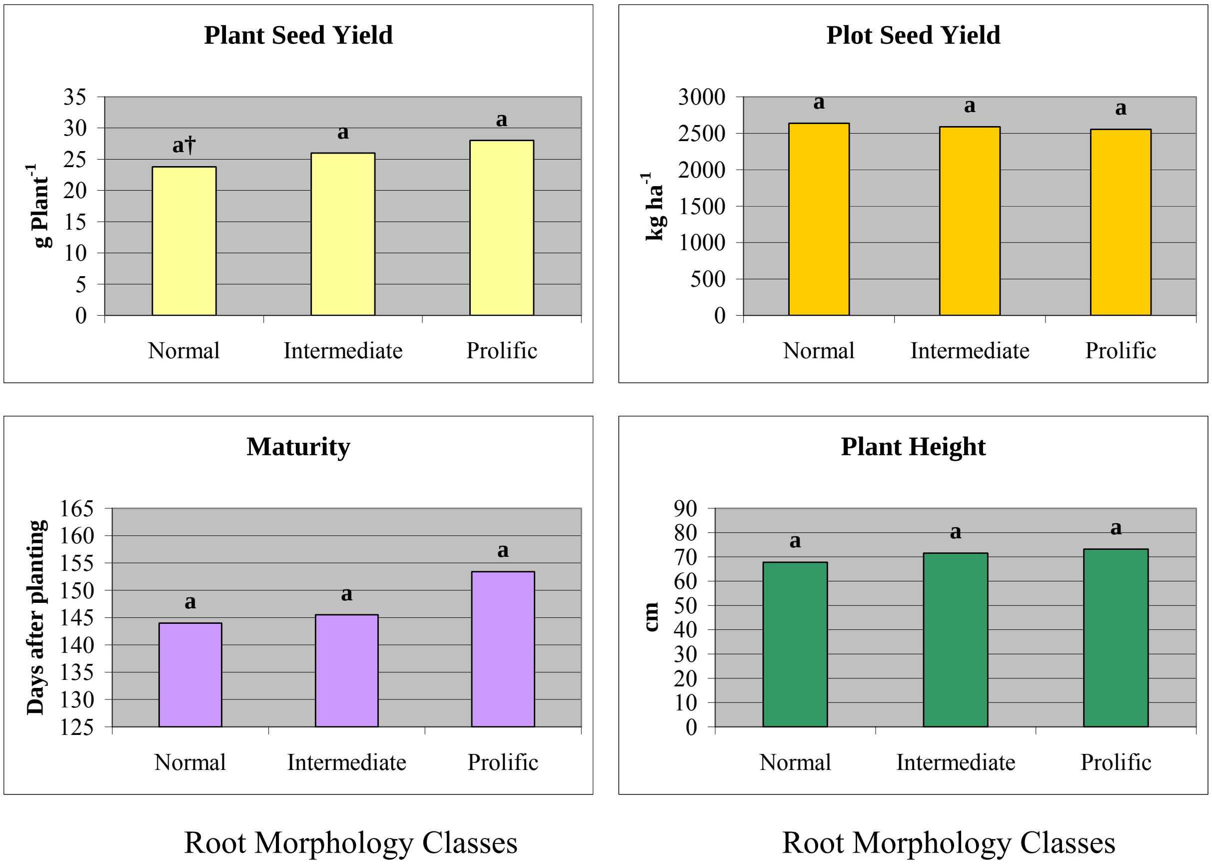

Figure 2.18. Comparison of plant seed yield, plot seed yield, maturity and plant height of 54 F4:6 and F7:8 population lines separated into classes of normal, intermediate, and prolific root morphology evaluated for two years, 2005-2006 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

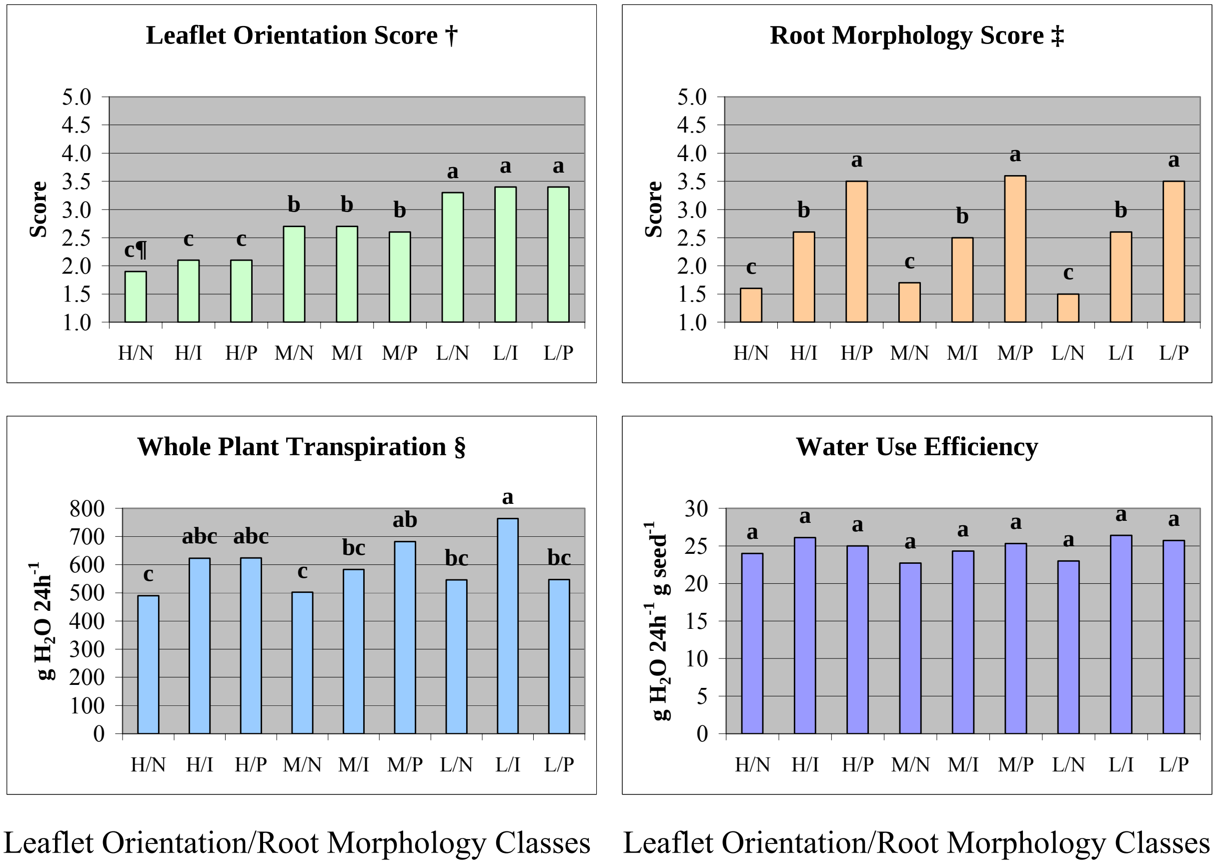

Figure 2.19. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 210 F4:6 population lines separated into nine combination classes consisting of high (H), medium (M), and low (L) leaflet orientation and normal (N), intermediate (I), and prolific (P) root morphology evaluated in 2005 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 2 August and 11 September, 2005.

¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

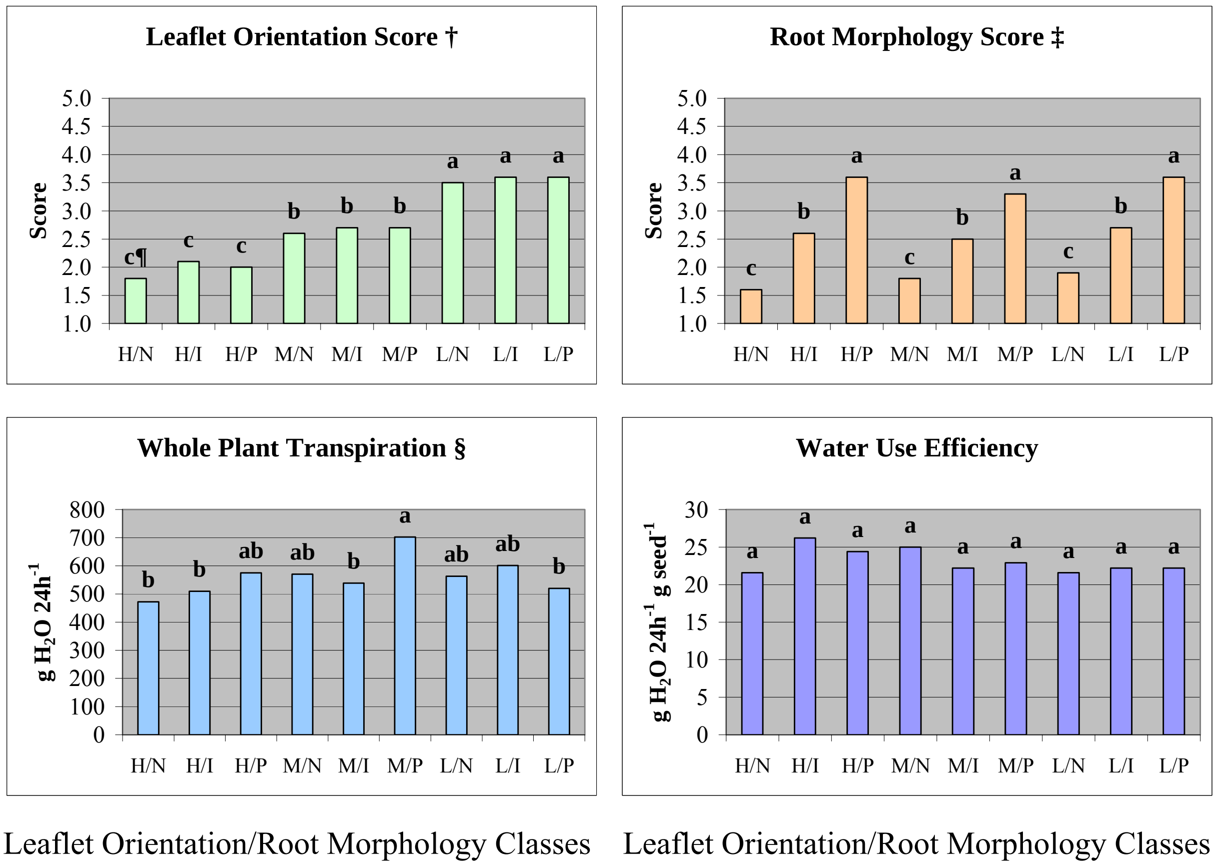

Figure 2.20. Comparison of leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 54 F4:6 and F7:8 population lines separated into nine combination classes consisting of high (H), medium (M), and low (L) leaflet orientation and normal (N), intermediate (I), and prolific (P) root morphology evaluated for two years, 2005-2006 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on two plants per line at R4 - R6 growth stage between the dates of 2 August and 11 September, 2005; measurements taken on four plants per line at R4 - R6 growth stage between the dates of 11 August and 15 September, 2006.with Dynamax Flow 32 Sap Flow Monitoring System ™.

¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

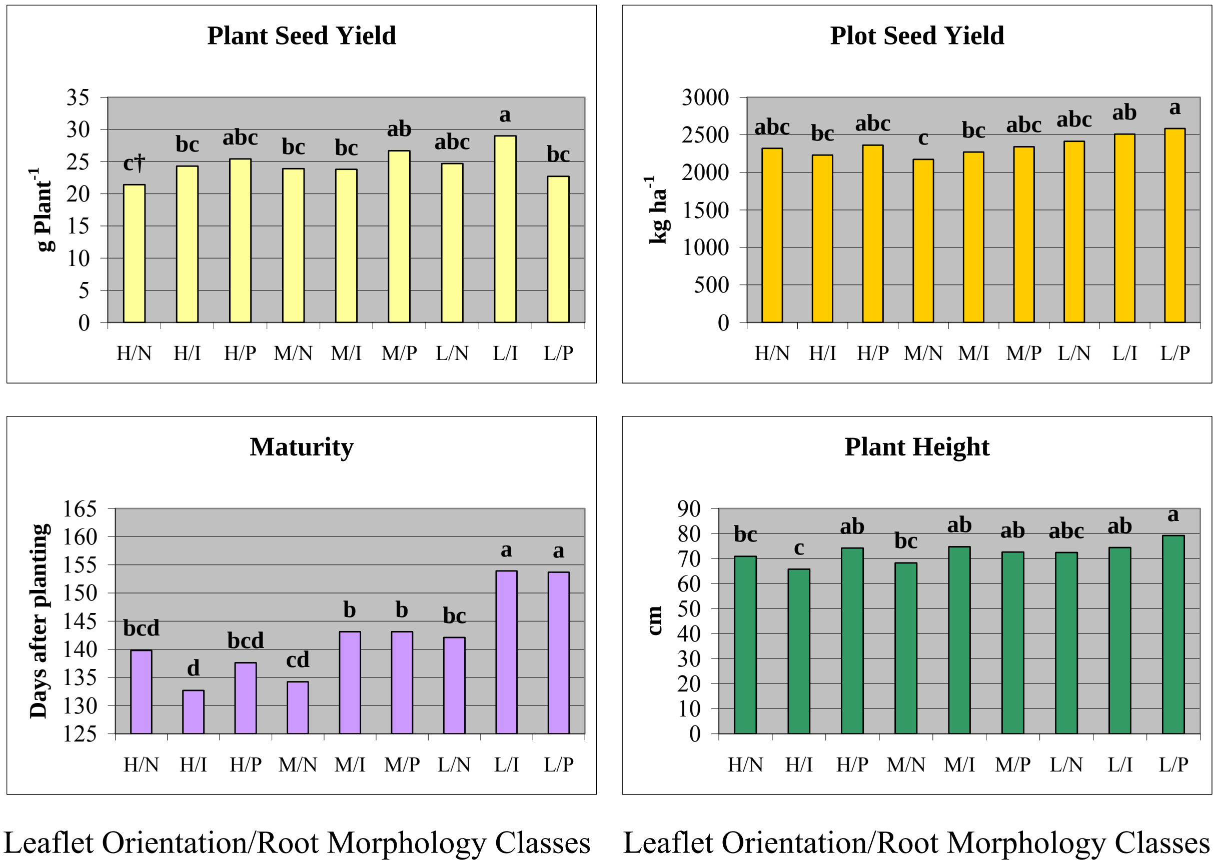

Figure 2.21. Comparison of plant seed yield, plot seed yield, maturity and plant height of 210 F4:6 population lines separated into nine combination classes consisting of high (H), medium (M), and low (L) leaflet orientation and normal (N), intermediate (I), and prolific (P) root morphology evaluated in 2005 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.
Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

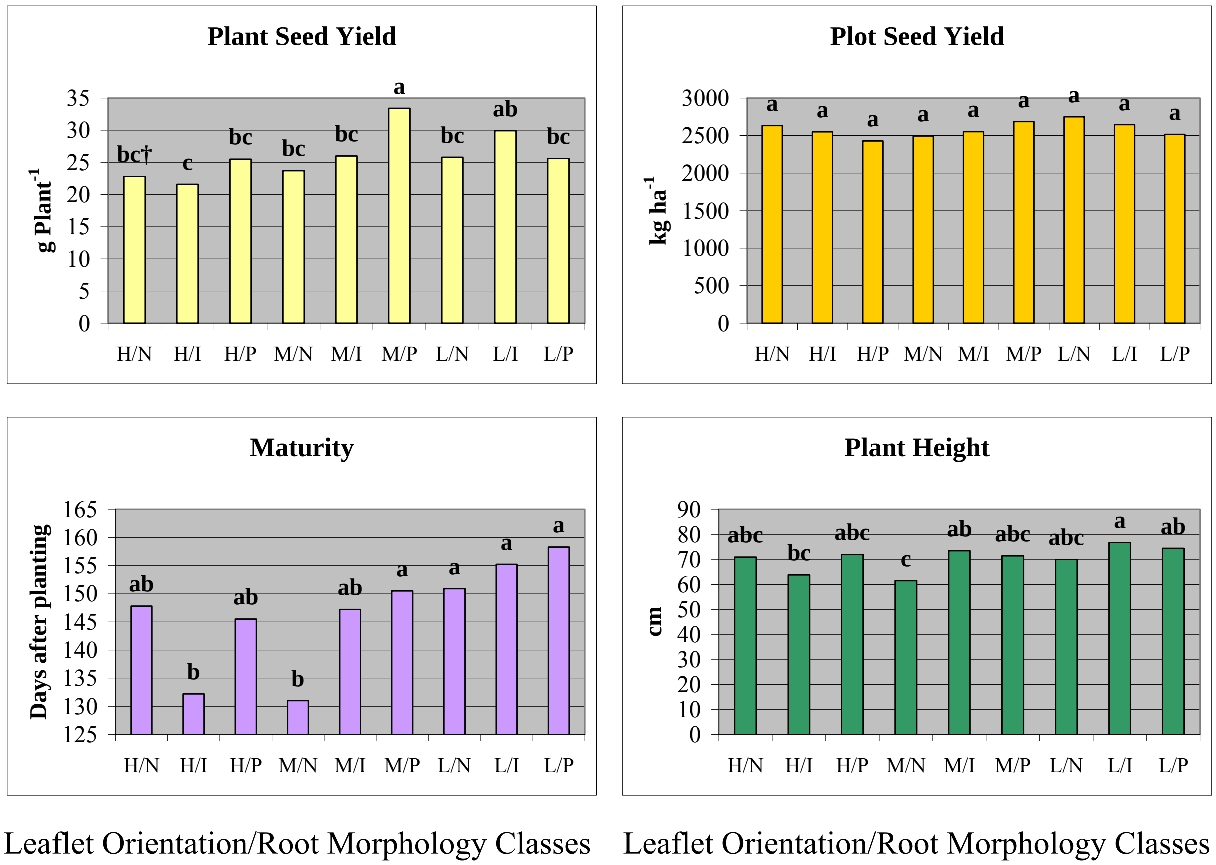

Figure 2.22. Comparison of plant seed yield, plot seed yield, maturity and plant height of 54 F4:6 and F7:8 population lines separated into nine combination classes consisting of high (H), medium (M), and low (L) leaflet orientation and normal (N), intermediate (I), and prolific (P) root morphology evaluated for two years, 2005-2006 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

Plot yield is average of four locations Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Part III

Effects of Leaflet Orientation and Root Morphology Traits on Transpiration, Yield and other Physiological Characteristics among Near-Isogenic Soybean Population Lines

Abstract

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress as it reduces global soybean yield by approximately 40%. The objective of this study was to evaluate the effects of two proposed drought tolerance traits, leaflet orientation and root morphology, on whole plant transpiration rates, yield, water use efficiencies and other agronomic traits in soybeans. Experiments were conducted across the state of Tennessee (USA) during the 2006 and 2007 growing seasons using 21 $F_{3:6}$, $F_{3:7}$, $F_{4:7}$, and $F_{4:8}$ near- isogenic line pairs. Growing conditions across the experimental locations in 2006 were characterized by abundant soil moisture while the 2007 growing season sustained record drought conditions. Whole plant transpiration rates, single plant yield, biomass production, leaf area, seed size, leaflet transpiration, stomatal conductance, photosynthesis rates, photosynthetically active radiation (PAR), solar radiation (SAR), soil moisture, leaflet orientation and root morphology scores were measured at Knoxville, TN USA (35.89 lat., -83.96 long.) on several successive days. Whole plant transpiration was measured on four plants of each line in each year of the study using the Dynamax Flow 32 Sap Flow Monitoring System when the plants were in the active pod filling stage of growth (R4-R6). The amount of water transpired by the treatment in a 24 hour period during seed fill was divided by the grams of seed produced by that plant in order to obtain estimates of water use efficiency. In order to evaluate yield and other agronomic traits, replicated plots were also planted at Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan, TN (35.93 lat., -88.70 long.). All data were analyzed using SAS Proc Mixed with the soybean lines considered as fixed effects and all other effects considered random in order to obtain least squares means of traits for each line for each

year and location. Least squares means of all lines were then used in the correlation and phenotypic class analyses. The current study detected no consistent patterns or significant effects due to differing leaflet orientation and root morphology scores among this set of near-isogenic lines for any of the measured traits. The current study was limited by the lack of prominent differences in leaflet orientation (1.0 on a 1.0 to 5.0 scale) and root morphology (0.9 on a 1.0 to 5.0 scale) between the near-isogenic line pairs. It is somewhat probable that this lack of more prominent differences affected the results of this study. Further study is needed to determine the effects of leaflet orientation and root morphology on whole plant transpiration, yield, water use efficiencies, and other agronomic characteristics in soybeans.

Chapter I

Introduction

The concept of ideotype breeding is basically identifying morphological traits that affect overall fitness, desirability and yield in a positive manner and then using those traits to assist in selection of superior performing genotypes (Donald, 1968). Ideotypes will vary depending on the species, environment, and overall goals of the breeding project. Many early studies involving this concept have involved canopy and root characteristics. Progress under this concept has been slow as many of the traits are complex and controlled by many genes. Their effects on yield can be small and therefore it is often difficult to prove a causal relationship. Additionally they may be linked to undesirable traits (Hamblin, 1993).

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress as it adversely affects total world soybean yield by approximately 40% (Pathan et al., 2007). Consequently, drought tolerance is a highly sought after trait in soybean cultivars. Drought tolerance is a complex response and is conditioned by the interaction of several genetic traits of the plant to environmental conditions (Chaves et al., 2003). Knowledge of these trait processes is needed not only for understanding plant resistance to drought stress but also to improve crop management and breeding techniques.

Many of the traits that are attributed to plant adaptation during drought such as phenology, root size and depth, and hydraulic conductivity are associated with plant development and structure and are constitutive rather than stress induced. A considerable part of plant resistance to drought is the ability to dissipate or avoid excess radiation. The nature of the mechanisms responsible for leaf photoprotection, especially those related to thermal dissipation and oxidative stress are therefore of great interest. A desirable plant type would be one that could endure drought conditions while maintaining a higher level of productivity by avoiding tissue dehydration, maintaining tissue water potential and photosynthesis as high as possible. Adaptive traits which condition dehydration avoidance include those which minimize excessive water loss and maximize water uptake. Water loss can be reduced by reducing light absorbance via steep leaf angles. Water uptake can be maximized by increasing the rooting volume and/or depth (Chaves et al., 2003). Thus, two potential traits of interest are leaflet orientation and root morphology. Leaflet orientation addresses the need to reduce water loss and root morphology addresses the ability to maximize water uptake.

Leaflet orientation

Many species of plants are capable of leaf movements in response to external stimuli (Ehleringer and Forseth, 1980). Leaf movement in response to light, known as heliotropism, can be classified as either diaheliotropic (light seeking) or paraheliotropic (light avoiding). Plants exhibiting diaheliotropism orient the plane of the leaf blade perpendicular to incident light rays, while plants exhibiting paraheliotropism orient the plane of the leaf blade parallel to incident light rays. Soybean exhibits both diaheliotropic and paraheliotropic movements, with the degree of movement being dependent on genotypic response (Wofford and Allen, 1982) and various levels of environmental stimuli (Ehleringer and Forseth, 1989; Rosa and Forseth, 1995).

Research conducted at the University of Tennessee demonstrated that soybean cultivars differ in their ability to orient leaflets during the course of the day (Wofford and Allen, 1982). Most cultivars exhibit high leaflet orientation (paraheliotropism) and move their leaves during the course of the day such that the leaves have maximum exposure to the sun in the early and late parts of the day, but during mid-day the leaves are oriented parallel to sunlight such that the surface of the leaves has minimum exposure to the sun. A lesser number of cultivars exhibit low leaflet orientation where the leaf surface remains relatively flat and changes little relative to the position and intensity of sunlight, even during the mid-day period of highest irradiance. These "low leaflet orienting" types are therefore relatively less paraheliotropic. In a study of the cultivar Essex (high leaflet orientation) and Dare (low leaflet orientation), the two cultivars produced about equal yields; however Essex used about one-half the amount of water as Dare during the growing season (Paris, 1997).

In work with soybean, Lugg and Sinclair (1981) found that upper leaflets of the canopy maintained a higher net photosynthetic rate per unit leaf area than did the lower leaflets. This seemed to be mostly due to shading, as the lower leaves were found to have photosynthetic rates similar to upper canopy leaves when unshaded. Kawashima (1969 a,b) found that soybean leaflets exhibiting paraheliotropism in the upper canopy allowed light to penetrate more deeply into the canopy, increasing photosynthetic output of the lower leaves, thus allowing total photosynthetic efficiency of the plant to be improved. Vertical leaf angles decrease the amount of solar radiation intercepted by the leaf. However photosynthetic rate response in plants to solar radiation is nonlinear and saturates below the intensity of direct ambient sunlight (van Zanten et al., 2010). Soybeans are reported to maximize their photosynthetic rates at less than one-third the amount of full sunlight according to Beuerlein and Pendleton (1971). Vertical leaflet orientation increases overall photosynthesis by allowing the upper canopy leaves to continue to photosynthesize under lower than ambient sunlight while also allowing lower canopy leaves to contribute at an increased rate (van Zanten et al., 2010).

Kao and Tsai (1998) studied leaf movements in three soybean species and found that paraheliotropism seemed to enhance water use efficiency and decrease the risk of photoinhibition in plants under water stress. Grant (1999) found that soybean plants that exhibit paraheliotropism are able to reduce UV-B irradiance in contrast to plants that do not orient leaflets. Ikeda and Matsuda (2002) studied photosynthetic efficiency differences in soybean leaves which were restrained from orienting versus naturally orienting. Their results indicated that paraheliotropic leaflet movements are an adaptation which optimizes net leaflet photosynthesis.

Isoda et al (1992, 1993) found that the paraheliotropic movements of soybean leaflets regulate light interception and reduce leaf temperature. Isoda and Wang (2002) studied leaf temperature and transpiration rates of cotton versus soybeans and found that soybeans were able to reduce leaf temperatures and transpiration rates. This was attributed to the soybean cultivars ability to orient its leaves in a paraheliotropic manner.

Leaflet orientation in soybeans has been related to increased light interception and yield potential (Shaw and Weber, 1967; Wang et al., 1995). However, Isoda and Tomagae (2003) compared biomass and seed yields of a highly orienting soybean cultivar which had its upper canopy leaves restrained from flowering to harvest in contrast to the same unrestricted cultivar. The study detected no differences in biomass or seed yields between the forced "low orienting" treatment and the "high orienting" control. There were also no differences detected in photosynthetic efficiencies or photoinhibition which may have been influenced by genotypic and/or environmental effects noted in the study as the results are contrary to previous research on the photosynthetic and photoprotective advantages of leaflet orientation (Shaw and Weber, 1967;

Prichard and Forseth, 1998; Ikeda and Mastuda, 2002; Wang et al., 1995; Jiang et al., 2006; Hirata et al., 1983; Rosa et al., 1991; Rosa and Forseth, 1995; Kao and Tsai, 1998).

Root Morphology

Development of breeding lines that have superior root systems may be an effective way to stabilize crop yields in drought-prone regions (Chaves et al., 2003; Kell, 2011). The ability of plants to resist drought has been found to be proportional to the density and extent of root development (Quizenberry, 1982). More expansive root architecture also allows plants to exploit soil mineral resources which may aid in increased nutrition, drought tolerance and yield (Lynch, 1995). A deeper and more expansive root system may allow soybean plants to efficiently access more soil area and thus more soil moisture (Pathan et al., 2007, Taylor, 1980). This might increase the ability of soybean plants to uptake water in drought stressed environments.

Significant variation for root size and morphology has been found in soybean (Quizenberry, 1982; Howard, 1980). Boyer et al. (1980) found that more recently developed, higher yielding soybean lines had lower mid-day water deficits and larger root densities than older, lower yielding cultivars. Garay and Wilhelm (1983) found that isolines of the soybean cultivar Harosoy which had greater root density, explored deeper into the soil profile and extracted more water during drought stress than the normal isoline. Jin et al. (2010) reported that a group of higher yielding soybean lines tended to have greater biomass, root mass and rooting depth than a group of lower yielding lines.

A soybean plant introduction cultivar from Japan, PI 416937 (Houjaku Kuwasu), which exhibits significant drought and aluminum tolerance (Goldman et al., 1989; Sloane et al., 1990; Hudak and Patterson, 1995) has been the focus of several researchers over the past 20 years.

This soybean line has also been characterized as possessing an extensive fibrous-like prolific root morphology which differs from the normal tap root of most soybeans (Hudak and Patterson, 1995; Pantalone et al., 1996a, 1999). Several studies have indicated the unique rooting morphology of PI 416937 as a major component of its ability to tolerate drought (Hudak and Patterson, 1995, 1996; Chipman et al., 2001). The prolific rooting morphology of the PI has been shown to support increased numbers of nitrogen fixing nodules (Pantalone et al., 1996a; Patterson and Hudak, 1996) and enhanced nitrogen fixation (Marlow, 1993) which may contribute to drought tolerance. The PI root system has also been shown to penetrate and continue to grow through hard soil layers that were impenetrable to other cultivars (Busscher et al., 2000). In addition to its root morphology, studies have indicated that PI 416937 may also tolerate drought by means of its osmotic regulation which appears to be somewhat different than that of other soybean cultivars. Fletcher et al. (2007) reported that PI 416937 demonstrated the ability to limit its transpiration rate under conditions of vapor pressure deficits associated with low humidity. Other genotypes continued to increase transpiration rates under increasing vapor pressure deficits. This contributes to the explanation of decreased soil desiccation by PI 416937 plants observed by Hudak and Patterson, (1996) and King et al. (2009).

Water Use Efficiency

Water use efficiency of crop plants can be improved by selection for improved transpiration efficiency and harvest index (Turner, 1993). Purcell (2006) stated the main tenets of crop physiology are that crop mass and yield are proportional to the cumulative amount of light intercepted and to the amount of water transpired by the crop during a season. Research indicates this to be true although the relationships may be more curvilinear than previously perceived. Edwards et al. (2005) found that although yield continued to increased with cumulative intercepted photosynthetically active radiation through 1100 MJ m^{-2}, 90% of maximum soybean yield can be obtained by intercepting 605 MJ m^{-2}. Similarly, Purcell et al. (2007) found that while soybean yield continued to increase with cumulative transpiration through 750 mm of soil profile water, 90% of the maximum yield could be obtained by transpiring 444 mm. This is encouraging for researchers who wish to improve soybean water use efficiencies as it indicates genotypes may exist, or can be developed, that regulate water use and light interception in such a manner as to maximize yield while using only as much water as needed. Identification of these types of plants and their associated traits would be of great interest to researchers and plant breeders.

Development and use of near isogenic lines

Near isogenic lines are useful genetic stocks for evaluating effects of genetically controlled traits. The goal of near isogenic line development is to create plants that are genetically and phenotypically similar in all respects except for the trait(s) of interest. Comparisons for effects can then be made between near isogenic genotypes which possess differing phenotypic attributes. Near isogenic lines are commonly produced by back crossing (Fehr, 1987). This method is best suited to genotypic traits controlled by one or few genes. Near isogenic lines can also be produced by descent and selection. This involves successive generations of inbreeding, usually through modified single seed descent methodology (Brim, 1966), in order to ensure some level of homozygosity and genetic similarity within each developed line. This is followed by detection and selection of those advanced homozygous lines which are still exhibiting segregation for the trait of interest (Haley et al., 1994; Yang et al., 1995; Mickelbart et al., 2003; Glover et al., 2004, Yamanaka et al., 2006). The development of near isogenic lines by descent can be particularly useful when dealing with a trait which is quantitatively controlled.

The objective of this research was to investigate the effects of leaflet orientation and root morphology on transpiration, seed yield, water use efficiency, biomass production and other physiological and agronomic traits of soybean by comparing these measured triats in near-isogenic lines sets which differ in the two traits of interest.

Chapter II

Materials and Methods

Experiments were conducted across the state of Tennessee (USA) during the 2006 and 2007 growing seasons using $F_{3:6}$, $F_{3:7}$, $F_{4:7}$, and $F_{4:8}$ near- isogenic line pairs in order to evaluate the effects of leaflet orientation and root morphology on transpiration, yield, water use efficiency, biomass, seed protein and oil production in soybean. Growing conditions across the experimental locations in 2006 were characterized by abundant soil moisture while the 2007 growing season sustained record drought conditions.

In the summer of 2002, 28 potential parental lines were planted at Knoxville, TN USA (35.89 lat., -83.96 long.) on an Etowah silt loam soil (fine-loamy, siliceous, semiactive, thermic, Typic Paleudult) and evaluated for leaflet orientation and root morphology differences. Twelve crosses were initiated July, 2002 in an attempt to create populations which contained significant and visually detectable levels of segregation for the two traits. The F1 seed of these crosses were grown in Costa Rica (Semillas Olson S.A., Costa Rica) during the months of November 2002 to April 2003 and evaluated for purity and correctness using the traits of flower and pubescence color. The F2 populations were grown during May to October at Knoxville on an Etowah silt loam soil (fine-loamy, siliceous, semiactive, thermic, Typic Paleudult) at which time a cross (USG 5601T × PI 416937) which contained the desired leaflet orientation and root morphology segregation patterns was identified. USG 5601T is a recently released (Pantalone et al., 2003) high yielding, maturity group V, determinate cultivar that exhibits high leaflet orientation and typical tap root morphology. PI 416937 is a maturity group VI, determinate plant introduction that exhibits low leaflet orientation and prolific fibrous-like root morphology (Pantalone et al.,

1999) (Figs. 3.1, 3.2). F2 plants were harvested and threshed separately at maturity using an Almaco BT-14 belt thresher (Almaco, Nevada, IA). F3 plants were advanced to the F4 generation by modified single seed descent (Brim, 1966) utilizing a winter nursery location in Homestead, FL (27 Farms, Homestead, FL) during the off season months of November 2003 through April 2004. F3 and F4 generation lines were planted and evaluated for leaflet orientation at Knoxville during the 2004 growing season as a good supply of remnant F3 seed was available. Sixty four F3 and their 64 corresponding F4 progeny lines (128 total F3 and F4 lines) were identified that appeared to be exhibiting continued segregation for leaflet orientation. No evaluation for root morphology segregation within lines was conducted at that time. Ten random single plant selections were advanced from each of the 64 F3 and 64 F4 line progeny rows to the F4 and F5 generations which were grown at Knoxville in 2005. The approximately 1280 F4 and F5 progeny rows were planted in continuous blocks arranged with each of the 10 sister line progeny rows planted next to each other. Each block of 10 sister progeny rows was evaluated for visually detectable differences in leaflet orientation. Progeny row lines were considered potential near isogenic pairs if they differed in leaflet orientation but were alike in all other visually detectable aspects and were sister progeny rows which were derived from the same 2004 F3 or F4 progeny row. Twenty six sets of potential near-isogenic line pairs differing in leaflet orientation were identified and selected for further development and evaluation. The resulting line pairs were advanced at winter nursery and evaluated as F3:6 and F4:7 in 2006 and as F3:7 and F4:8 in 2007 at Knoxville. Only the line pairs that consistently differed in trait expressions of leaflet orientation and root morphology were deemed as near-isogenic and used in the analyses of this study. This resulted in 12 near-isogenic line pairs for the trait of leaflet orientation and 15 near-isogenic line pairs for the trait of root morphology; however six lines were common to both sets, thus there was a total of 21 unique line pairs (Table 3.1). These F3 and F4 derived near-isogenic sister line pairs were therefore developed by descent and selection and are genetically similar at the 75% and 87.5% levels of homozygosity, respectively. Due to the suspected quantitative nature of both traits, the differences between the near-isogenic line pairs for these traits were not of great magnitude. Some lines developed from this population exhibited substantial shattering similar to the PI 416937 parent. There were three near-isogenic pairs (six lines) in this study that exhibited varied levels of shattering depending on the location and year. Yield data that seemed significantly impacted by shattering were excluded from the analyses.

Leaflet orientation score for each plot was taken on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining and angle parallel to the horizontal plane (Figure 3.1). Three replications of leaflet orientation scores were taken on 16 August, 25 August, and 16 September in 2006 and on 9 August, 20 August and 3 September in 2007 concurrent with the measurement period of whole plant transpiration. Leaflet orientation was rated between the hours of 1300 and 1500 for each replication as this is the period of the day in which the differential leaflet orientation was at its highest (Wofford and Allen, 1982).

Root morphology scores were obtained in 2006 and 2007 by removing the root system of intact plants from the soil and visually rating each set of plants for the phenotype in a similar manner described by Pantalone et al. (1996a). Root morphology scores were obtained visually using a soil inverter blade to loosen the soil and expose the root systems of hill plots planted each year in three replications when plants were in the R4 to R6 stage of growth on 25 August, 2006 and 20 August, 2007. Plots were planted in a slightly sandy, very friable Staser Silt Loam soil (fine-loamy, mixed, active, thermic, Cumulic Hapludoll) which facilitated soil removal and evaluation. Root morphology score is a phenotypic rating on a scale of 1 to 5 with 1 being the condition of the plant possessing a normal tap root with few lateral roots and 5 being the condition of the plant possessing a prolific root mass with many fibrous-like lateral branching roots (Figure 3.2).

Whole plant transpiration rates were measured at Knoxville on several successive days using the Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stage of growth (R4-R6). These whole plant transpiration rates were assumed to be representative of each line and parent during the reproductive stage of R4-R6. Although this measurement may not be representative of transpiration over the growing season, it is deemed important as it represents the period in which seed yield and seed quality constituents are developed and water use is at or near its peak (Wilson, 2004; Heatherly and Elmore, 2004). Consequently, this is also the approximate period when leaflet orientation values were found to be at their highest by Wofford and Allen (1982). Dynamax model SGA9 Flow32 System Dynagauges were used to connect each plant to the system as the approximate 9mm diameter size of the Dynagauge would properly fit around the lower stem of the R4-R6 soybean plants. Each plant was marked with a durable tag for identification purposes later in the season. The stem diameters were measured and cleaned. The interior of the Dynagauge sensor was lubricated with a very thin film of Dow Corning 4 Electrical Insulating Compound (Dow Corning Corp, Midland, MI) and then placed around the stem in such a manner as to ensure that the thermocouples and heater strip of the sensor were in direct contact with the stem. The top and bottom of the sensor was then sealed with Elmer's Poster Tack adhesive putty (Elmer's Products Inc., Westerville, OH). The sensor was then wrapped with a sheet of Reflectix double reflective insulation (Reflectix Inc., Markleville, IN) measuring approximately 14 cm x 33 cm which provides two layers of insulation. The insulation was held in place by placing a cable tie near the top, bottom and middle of the sensor in a manner such that the insulation was secure but with minimal pressure being applied to the stem. The system was mounted to a vertical cart with wheels for easier transportation within the field. The battery and data cables were placed in a large tool box also mounted to the cart. Additionally a solar panel was attached to the cart to extend the battery life and operating capacity of the system (Fig. 3.3).

In each year of the study, whole plant transpiration data were collected on four plants of each line over a period of two to four days depending on environmental conditions. The goal was to collect data from a 24 hour period when the conditions were mostly sunny; therefore some measurements covered a longer period of time due to cloudy days after the system was installed on the plant material. Transpiration data (grams of water per 24 hour period) from a single mostly sunny day from each set of measurements were used in this analysis. Data collected on other days were not utilized due to factors such as sensor malfunctions and/or environmental conditions. Whole plant transpiration data were collected between 19 August and 21 September, 2006 and on 8 August and 7 September, 2007. The maximum capacity of the Dynamax Flow 32 Sap Flow Monitoring System was 32 sensors. Therefore, only eight genotypes could be measured during any single measurement period. In order to adjust the measurements for variations due to differing environmental conditions between days, each set of measurements included the parents.

The plants which were tagged and measured for whole plant transpiration were harvested and threshed at maturity using an Almaco BT-14 belt thresher (Almaco, Nevada, IA) in order to obtain single plant yields. The amount of water transpired by the treatment in a 24 hour period during seed fill was divided by the grams of seed produced by that plant in order to obtain an estimate of water use efficiency.

Plant material in all experiments were seeded using a commercial planter (John Deere, Max Emerge, Moline, IL) equipped with plot cone seeding units (model CTS, Almaco, Nevada, IA). All plants were seeded at a density of approximately 3 cm apart into double row plots which were 6 m in length with 76.2 cm spacing between each row. In addition to plots at Knoxville which were used to measure physiological traits, additional research plots were planted in order to evaluate yield and agronomic traits such as maturity, height, lodging, shattering and seed protein and oil. These locations included Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan, TN (35.93 lat., -88.70 long.). All plots at Knoxville during 2004 – 2007 were planted on a Staser silt loam soil (fine-loamy, mixed active, thermic Cumulic Hapludoll). Yield trial plots at Springfield were planted on a Dickson silt loam soil (fine-silty, siliceous, semiactive, thermic Glossic Fragiudult) and a Mountview silt loam soil (fine-silty, siliceous, semiactive, thermic Oxyaquic Paleudult) in 2006 and 2007, respectively. Yield trial plots at Spring Hill were planted on a Maury silt loam soil (fine, mixed, active, mesic Typic Paleudalf). Yield trial plots at Milan, TN USA (35.93 lat., -88.70 long.) were planted on a Falaya silt loam soil (coarse-silty, mixed, active, acid, thermic Aeric Fluvaquent) and a Loring silt loam soil (fine-silty, mixed, active, thermic Oxyaquic Fragiudalf) in 2006 and 2007, respectively. Each yield trial entry was replicated two or three times at each location in 2006 and 2007, respectively. Yield trial plots were harvested at all locations with an Almaco SPC 40 plot combine (Almaco, Nevada, IA).

In 2006, four plants from each near-isogenic line were collected concurrent with the transpiration measurement in order to obtain values of leaf area and biomass. Entire plants, including roots, were removed from the field and immediately weighed. The plants were then defoliated and the leaf area measured using a Delta T area meter (Delta T Devices, Cambridge, England). All plant parts were then dried in a forced-air dryer at a temperature of 54.4°C for approximately 96 hours or until such time as the weight of the sample stabilized. Samples were then weighed in order to obtain estimates of dry weight biomass production.

Photosynthetically active radiation (PAR), solar radiation (SAR), and soil moisture were recorded at the Knoxville location using a Hobo® weather station equipped with H21-001 data logger, S-LIA-M003 PAR, S-LIB-M003 pyranometer, and S-SMA-M003 soil moisture sensors (Onset Computer Corporation, Pocasset, MA).

In 2006, leaflet transpiration, stomatal conductance, and photosynthesis data were obtained from an upper canopy leaflet of each line which was exposed to full sunlight using the Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX).

Protein and oil analysis was performed on a Foss Model 1229 NIR analyzer (Foss NIRSystems Inc., Laurel, MD).

All data were analyzed using SAS Proc Mixed with the near-isogenic lines and parents considered as fixed effects and all other effects considered random. Least squares means with mean separation and average LSD values were obtained using the SAS macro written by Saxton (1998) for all measured traits for each line in each year and location. In addition, the least squares means data were used to analyze the effects of leaflet orientation and root morphology on other measured traits. This was accomplished by separating the data into higher and lower leaflet orientation classes as well as more and less prolific root morphology classes. These least squares means data sets were analyzed using SAS Proc GLM as model effects were previously adjusted and only independent and dependent variables remained (SAS User Guide 9.1.3, 2006).

Chapter III

Results and Discussion

Each of the lines within each of the 12 line pairs deemed near-isogenic for leaflet orientation differed from each other in leaflet orientation score significantly, however the differences were not of great magnitude. Most of the near-isogenic pairs differed by an average leaflet orientation score of 0.9 on a 1 to 5 scale. Observation and phenotypic data collected over the three year selection and evaluation period, confirmed the overall genetic similarity of each line pair near-isogenic for leaflet orientation. Although there were significant differences between near-isogenic line sets for all measured traits, no significant differences were detected within any of the near-isogenic sets between line pairs for maturity, plant height, and leaf area. However, near-isogenic lines within three of the line pairs had significantly differing root morphology scores, one pair differed in lodging score, four pairs differed in seed size, two pairs differed in seed protein and one pair differed in seed oil (Table 3.2) Six of the line pairs deemed near-isogenic for leaflet orientation were also considered near-isogenic for root morphology. These include the three line pairs near-isogenic for leaflet orientation that differed significantly in rooting score (Sets 6, 9, 23) (Table 3.1) and three additional line pairs which although not significantly different for rooting score, were consistently different for root score across the period of evaluation (Sets 2, 18, 21).

Slightly higher root morphology scores were associated with lower leaflet orientation in nine of the 12 isogenic line sets; however only two of these differences between isogenic pairs were statistically significant (Sets 6, 11) (Table 3.2). A third significant difference was detected, however in that case (Set 23) the lower leaflet orientation line had a lower root morphology score

(Table 3.2). There were no differences detected in root morphology between the 12 higher and 12 lower leaflet orientation near-isogenic line sets when analyzed and compared as separate classes (Table 3.3, Fig. 3.6). Thus no pattern of association was detected between leaflet orientation and root morphology in this study.

Transpiration in the soybean plants during the monitoring period appeared to began at approximately 0800 h, reaching a peak at approximately 1500 h, and ceasing at approximately 2000 h (Fig. 3.4). While transpiration curves differed in overall magnitude, they were somewhat similar in shape within a day of measurement. There were variations in the overall shapes of the transpiration curves between days due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration. The similarities observed between the transpiration curves and the PAR curves within each day demonstrates the close relationship between PAR and transpiration (Fig 3.5). The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis and transpiration. These observations are similar to those reported previously for soybean by Gerdes et al. (1994).

There were no discernable trends detected between the traits of leaflet orientation and whole plant transpiration. Two statistically significant differences were detected within the near-isogenic line pairs. However, these differences were contradictory as in set 2, the higher transpiration rate (909 g H2O $24h^{-1}$ vs. 581 g H2O $24h^{-1}$) was associated with the higher leaflet orientation line while in set 6 the higher transpiration rat was associated with the lower leaflet orientation near-isogenic line (497 g H2O $24h^{-1}$ vs. 704 g H2O $24h^{-1}$) (Table 3.2). There were no differences detected in whole plant transpiration rates between the 12 higher and 12 lower leaflet orientation sets when analyzed as separate classes (Table 3.3, Fig. 3.6). This is somewhat contradictory to previous research which indicates that paraheliotropic leaf movements lower both leaflet temperatures and leaflet transpiration (Prichard and Forseth, 1988; Bielenberg et al., 2003; Kao and Forseth, 1992; Yu and Berg, 1994; Isoda and Wang, 2001, 2002; Wien and Wallace 1973; Shackel and Hall, 1979; Meyer and Walker, 1981; Berg and Hsiao, 1986; Forseth and Teramura, 1986; Berg and Heuchelin, 1990) This may be due to the lack of more prominent differences between the leaflet orientation scores of the near-isogenic lines.

No association was detected between leaflet orientation and yield. Five statistically significant differences were detected within the near-isogenic line pairs for single plant yield. However, these were not consistently associated with higher or lower leaflet orientation (Table 3.2). There were no differences detected in single plant yield or plot yield between the 12 higher and 12 lower leaflet orientation sets when analyzed separately as a class (Table 3.3, Fig. 3.7). These results conflict somewhat with observations by other researchers which indicate that by orienting upper canopy leaves, plants allow more light to penetrate into the lower canopy which may increase the overall amount of light interception and photosynthesis of the plant. (Kawashima, 1969 a,b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000; Isoda and Wang, 2001). Such an increase in photosynthesis may be associated with increased yield (Wang et al., 1995; Chang and Taqumpay, 1970; Mickelson et al., 2002; Pendelton et al., 1968; Pepper et al., 1977).

No differences were detected for water use efficiency within any of the near-isogenic pairs nor between the leaflet orientation phenotypic classes. This finding is to be somewhat expected since no differences were detected between leaflet orientation classes for transpiration and yield. Similarly no consistent differences were detected for maturity, plant height, lodging, seed size, seed protein, seed oil, dry weight biomass, or leaf area within any of the near-isogenic line pairs nor between the leaflet orientation classes (Tables 3.2, 3.3, 3.4, 3.5, Figs. 3.6, 3.7). Additionally no differences were detected between high and low leaflet orientation classes for the traits of transpiration, stomatal conductance, and photosynthesis rates of upper canopy leaflet tissue exposed to full sunlight (Table 3.5). This indicates that the two classes of leaflet orientation were somewhat equal in their ability to transpire and photosynthesize. Any differences therefore that might have been detected could have been more related to leaflet orientation differences than to differences in plant leaflet traits.

The 15 line pairs deemed near-isogenic for root morphology in this study were chosen based on their consistent differences in root morphology scores in each year over the two year period. However, the differences in root morphology scores for the 15 near-isogenic line pairs were not of great magnitude. Only two of the 15 line pairs near-isogenic for root morphology were found to contain lines within the pairs that differed significantly from each other in root score (Table 3.7). These were set 6 (with root scores of 1.8 and 3.0) and set 23 (with root scores of 1.5 and 3.0). However, when the 15 more prolific and 15 less prolific root morphology near-isogenic line sets were analyzed as separate classes, they were found to differ significantly (Table 3.7). Observation and phenotypic data collected confirmed the overall genetic similarity of each line pair near-isogenic for the root morphology trait. Although there were significant differences between near-isogenic line sets for all measured traits, no significant differences were detected between lines within any of the near-isogenic sets pairs for maturity, plant height, and leaf area. However, nine of the pairs had significantly differing leaflet orientation scores, one pair differed in lodging score, six pairs differed in seed size, one pair differed in seed protein and one pair differed in seed oil (Table 3.6).

There were no consistent differences or trends to indicate association between rooting score and leaflet orientation. While nine of the 15 near-isogenic pairs contained lines which differed significantly for leaflet orientation score, four of these had lower leaflet orientation associated with more prolific rooting and five had lower leaflet orientation associated with less prolific rooting (Table 3.6). For example, set 6 contains line 60502-2 which is a more prolific rooted line with lower leaflet orientation relative to the other near-isogenic line in that set, 60502-10. Set 23 on the other hand, contains line 70685-5 which is a less prolific rooted line with higher leaflet orientation. There was no difference detected in leaflet orientation between the 15 more prolific and 15 less prolific root morphology near-isogenic line sets when analyzed as separate classes (Table 3.7, Fig. 3.8). Thus no pattern of association was detected between leaflet orientation and root morphology in this study.

There were no discernable trends detected between the traits of root morphology and whole plant transpiration. Four statistically significant differences were detected within the near-isogenic line pairs (Sets 2, 6, 15, 26) (Table 3.6). However, these differences were contradictory as three of the higher transpiration rates were associated with less prolific root morphology while the other was associated with more prolific root morphology. There were no differences detected in whole plant transpiration rates between the 15 more prolific and 15 less prolific phenotypic class sets (Table 3.7, Fig. 3.8). Previous studies have suggested that the unique prolific rooting morphology of PI 416937 may be a major component of its ability to tolerate drought (Hudak and Patterson, 1995, 1996; Chipman et al., 2001). Other studies have indicated that PI 416937 tolerates drought by means of limiting transpiration via osmotic regulation which decreases soil moisture loss throughout the growing period (Fletcher et al., 2007; Hudak and Patterson, 1996; King et al., 2009). The contribution of root morphology on this observed decrease in transpiration was previously unknown. In part I of this dissertation, the effect of rooting morphology on whole plant transpiration rates were investigated via grafting and no differences were found betweem root phenotypic classes of prolific versus normal. Although the prolific root phenotype may aid drought tolerance for other reasons, the current study supports previous findings that indicate it has a more limited role in transpiration rate modification. However, the results of the current study may also be due to the lack of more prominent differences between the root morphology scores of the near-isogenic lines.

There were no detectable associations between root morphology and yield. Four statistically significant differences were detected within near-isogenic line pairs for single plant yield. However, these were not consistently associated with more prolific or less prolific root morphology (Table 3.6). There were no differences detected in single plant yield or plot yield between the class sets (Table 3.7, Fig. 3.9). Previous studies have suggested a positive relationship between yield and increased root mass (Hammer et al., 2009; Lopes and Reynolds, 2010; Boyer et al., 1980; Jin et al., 2010). However, Pantalone et al. (1996b) found a non-significant but negative correlation ($r = -0.56$) between prolific rooting and plot yield. Part II of the current dissertation study found positive correlations between root morphology and single plant yield ($r=0.27$, $p=0.04$) but no association with plot yield ($r=0.05$, $p=0.69$). The current study indicates little to no effect of differing root morphology scores on yield.

Water use efficiencies were not significantly different between the near-isogenic root morphology phenotypic class sets (Table 3.7, Fig. 3.8). Only three of the 15 near-isogenic pairs contained lines which differed significantly, however there was no consistency or pattern of association between water use efficiencies and the root morphology phenotype. This finding is to be somewhat expected since no differences were detected between root morphology classes for transpiration and yield. Similarly no consistent differences were detected for maturity, plant height, lodging, seed size, seed protein, seed oil, dry weight biomass, or leaf area within any of the near-isogenic line pairs or between the leaflet orientation classes (Tables 3.6, 3.7, 3.8, 3.9 Figs. 3.8, 3.9). Pantalone et al. (1996b, 1999) found positive correlations between root score and seed protein although the correlations were not always significant. Pantalone et al. (1996b) found negative correlations between prolific rooting scores and seed oil content and positive correlations between root morphology scores and seed size, although the effects were not always significant. Previous studies have found positive relationships between root mass and biomass accumulation (Pantalone et al., 1999; Hammer et al., 2009; Jin et al., 2010). The current study contradicts those previous studies, however, the results of this study may be confounded by the lack of more prominent differences between the root morphology scores of the near-isogenic lines.

No differences were detected root morphology classes for the traits of transpiration, stomatal conductance, and photosynthesis rates of upper canopy leaflet tissue exposed to full sunlight (Table 3.9). This indicates that the plants contained in the two classes of root morphology were somewhat equal in their ability to transpire and photosynthesize. Any differences therefore that might have been detected could have been more related to root morphology differences than to differences in plant leaflet traits.

Chapter IV

Conclusions

The objective of this study was to investigate the effects of leaflet orientation and root morphology on transpiration, seed yield, water use efficiency, biomass production and other physiological and agronomic traits of soybean by comparing these measured triats among near-isogenic lines sets which differed in the two traits of interest.

Based on previous research it was thought that higher leaflet orientation might result in lower transpiration rates (Meyer and Walker, 1981; Isoda and Wang, 2001), higher yields (Wang et al., 1995; Mickelson et al., 2003) and better overall water use efficiencies (Kao and Tsai, 1998; Bielenberg et al., 2003). Additionally it has been proposed that prolific rooting morphology might influence transpiration rates (Hudak and Patterson, 1996), ability of plants to access more soil moisture under drought conditions (Sloane et al., 1990; Hudak and Patterson, 1995), as well as increase yields (Jin et al., 2010), seed protein levels and biomass accumulation (Pantalone et al., 1996b, 1999).

Pairs of near-isogenic lines for phenotypic traits of leaflet orientation and root morphology were developed by descent from the cross of USG 5601T and PI 416937. Closely related sister lines that seemed to exhibit segregation primarily for the traits of interest were selected as single plants in the F3 and F4 generations for evaluation, selection and development. The resulting line pairs were advanced at winter nursery and evaluated as F3:6 and F4:7 in 2006 and as F3:7 and F4:8 in 2007 at Knoxville. Only the line pairs that consistently differed in trait expressions of leaflet orientation and root morphology over the two year period of the study were deemed as near-isogenic and used in the analyses of this study. This resulted in 12 near-isogenic line pairs for the trait of leaflet orientation and 15 near-isogenic line pairs for the trait of root morphology; however six lines were common to both sets, thus there was a total of 21 unique line pairs (Table 3.1). Due to the suspected quantitative nature of both traits, the differences between the near-isogenic line pairs for these traits were not of great magnitude.

The current study detected no consistent patterns or significant effects due to differing leaflet orientation and root morphology scores among this set of near-isogenic lines for any of the measured traits. Hamblin (1993) stated that progress related to studies investigating effects of canopy and root characteristics on yield and other traits has been slow as many of the traits are complex and controlled by many genes. Their effects may also be small which can result in difficulty in proving a causal relationship. The current study may have been limited by the lack of prominent differences in leaflet orientation and root morphology between the near-isogenic line pairs. Similarly there was an overall lack of prominent differences between the higher and lower leaflet orienting sets when separated and analyzed as separate classes. The means of the two classes differed significantly, however this was only a difference of 1.0 on a 1 to 5 scale (Table 3.3). This was also the case for the more and less prolific root morphology classes. It is likely that this lack of more prominent differences affected the results of this study. Providing that resource limitations allow, perhaps larger sampling from each of the F3 and F4 families during near-isogenic line development would have resulted in line pairs with greater differences and enhanced the current study. It is therefore suggested that further study is needed to determine the effects of leaflet orientation and root morphology on whole plant transpiration, yield, water use efficiencies and other agronomic characteristics in soybeans. Development of superior near-isogenic line pairs could be attempted by selecting larger numbers of single plants from advanced generation segregating population lines. Selection of segregating lines in the F4, F5, and/or F6 geneations along with the advancement and evaluation of 50 – 80 single plant harvested progeny rows, might result in detection and development of near-isogenic pairs with more prominent phenotypic differences than were found in the current study.

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Trade names or commercial products were mentioned solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the University of Tennessee.

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APPENDIX

Part III

Table 3.1. Twenty six near-isogenic line sets evaluated for potential differences in leaflet orientation and root morphology during 2006 and 2007 in Knoxville, TN from which 12 F3:6 and 30 F4:7 lines were selected for further evaluation.

Near Isogenic Line Set	Line	Relative Leaflet Orientation Class	Leaflet orientation			Relative Root Mophology Class	Root morphology		
			2006	2007	2 yr		2006	2007	2 yr
				(score)†				(score)‡	
1	60038 - 2	---	3.0	2.4	2.7	---	2.0	3.5	2.6
	60038 - 7	---	2.5	3.3	2.9	---	2.7	2.7	2.7
2	60115 - 2	H §	2.3 *	2.6 *	2.4 *	N	1.7	1.3	1.5
	60115 - 8	L	3.0 *	3.4 *	3.2 *	P	2.3	2.7	2.5
3	60121 - 8	---	2.2	3.6	2.9	---	2.7	2.0	2.3
	60121 - 9	---	3.2	2.9	3.0	---	2.7	1.7	2.2
4	60247 - 6	---	2.3	3.4	2.9	---	1.7	3.0	2.3
	60247 - 8	---	3.7	2.7	3.2	---	3.3	3.0	3.2
5	60259 - 10	H	2.3 *	2.4 *	2.4 *	---	3.3	1.7	2.5
	60259 - 7	L	3.3 *	3.8 *	3.5 *	---	3.0	3.3	3.2
6	60502 - 10	H	2.3 *	2.8 *	2.6 *	N	2.3	1.3 *	1.8 *
	60502 - 2	L	3.5 *	3.7 *	3.6 *	P	2.7	3.3 *	3.0 *
7	60512 - 5	---	2.5	3.6	3.0	---	2.0	1.7	1.8
	60512 - 9	---	3.8	2.6	3.2	---	2.0	1.3	1.7
8	60537 - 10	---	2.8	3.4	3.1	N	2.0	2.3	2.2
	60537 - 9	---	3.5	2.6	3.0	P	3.0	3.0	3.0
9	60558 - 6	L	3.3 *	4.0 *	3.7 *	P	3.0 *	2.3	2.7
	60558 - 9	H	2.5 *	2.8 *	2.6 *	N	1.7 *	2.0	1.8
10	60613 - 5	---	3.0	3.8	3.4	N	3.0	2.7	2.8
	60613 - 8	---	3.0	2.7	2.9	P	3.7	3.7	3.7
11	70094 - 10	L	3.8 *	4.0 *	3.9 *	---	2.0	3.7	2.8
	70094 - 9	H	2.5 *	3.0 *	2.7 *	---	2.0	1.3	1.7
12	70115 - 8	---	2.3	2.8	2.5	---	3.0	1.0	2.0
	70115 - 9	---	2.5	2.2	2.3	---	3.0	1.3	2.2
13	70139 - 10	L	3.5 *	3.6 *	3.5 *	---	3.0	2.0	2.5
	70139 - 8	H	2.5 *	2.8 *	2.7 *	---	2.3	2.7	2.5
14	70175 - 2	---	2.0	2.5	2.2	N	3.7	1.7	2.7
	70175 - 7	---	2.5	2.6	2.5	P	4.0	2.7	3.3
15	70247 - 2	---	2.5	3.6	3.0	N	2.7	3.0	2.8
	70247 - 6	---	3.0	2.6	2.8	P	3.3	4.3	3.8
16	70259 - 5	---	2.5	3.5	3.0	N	3.0	2.3	2.7
	70259 - 7	---	2.5	2.6	2.5	P	3.3	3.3	3.3
17	70297 - 10	L	3.0 *	3.6 *	3.3 *	---	2.0	2.7	2.3
	70297 - 9	H	2.0 *	2.3 *	2.2 *	---	2.0	1.7	1.8
18	70471 - 6	H	2.2 *	2.4 *	2.3 *	N	2.7	1.7	2.2
	70471 - 7	L	2.8 *	3.3 *	3.0 *	P	3.3	3.0	3.2
19	70500 - 2	---	2.5	3.7	3.1	P	4.0	2.3	3.2
	70500 - 5	---	3.0	2.9	3.0	N	3.3	2.0	2.7
20	70558 - 2	---	3.3	3.7	3.5	N	1.7	1.7	1.7
	70558 - 8	---	3.3	3.2	3.2	P	2.3	2.3	2.3
21	70624 - 3	H	2.5 *	2.7	2.6 *	P	2.0	3.7	2.8
	70624 - 6	L	3.5 *	3.3	3.4 *	N	1.7	2.0	1.8
22	70638 - 2	---	2.8	3.3	3.0	P	2.0	3.3	2.7
	70638 - 6	---	3.3	2.6	2.9	N	1.0	2.7	1.8
23	70685 - 4	H	2.0 *	2.6	2.3 *	P	3.0	3.0 *	3.0 *
	70685 - 5	L	3.5 *	3.2	3.3 *	N	2.0	1.0 *	1.5 *
24	70685 - 8	L	3.3 *	3.8 *	3.6 *	---	2.3	1.7	2.0
	70685 - 9	H	2.5 *	2.5 *	2.5 *	---	2.3	1.3	1.8
25	70821 - 6	L	3.3 *	3.4 *	3.4 *	---	3.0	3.0	3.0
	70821 - 9	H	2.5 *	2.7 *	2.6 *	---	2.0	3.3	2.7
26	70934 - 2	---	2.5	3.3	2.9	---	2.0	1.0	1.5
	70934 - 5	---	2.0	2.6	2.3	P	2.0	2.3	2.2
	70934 - 6	---	3.2	2.3	2.7	N	1.7	1.3	1.5
$Pr>F_{.05}$			<0.0001	<0.0001	<0.0001		<0.0001	0.0045	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = denotes 12 near-isogenic line pairs selected for contrasting leaflet orientation and 15 pairs selected for contrasting root morphology with 6 lines being common to both sets; these lines differed in leaflet orientation and/or root morphology scores in a consistent manner over the two year evaluation period were deemed near-isogenic for the traits of leaflet orientation and/or root morphology and used in analyses.
* = denotes that mean separation difference at the α = 0.05 level of significance (only noted for confirmed near-isogenic pairs).

Table 3.2. Comparison of leaflet orientation, root morphology, whole plant transpiration, water use efficiency, single plant yield, plot yield, maturity, lodging, seed size, protein, oil, dry weight and leaf area of 12 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of higher and lower leaflet orientation evaluated in 2006 and 2007 in Tennessee.

Near isogenic line set	Line	Relative Leaflet Orientation	Leaflet orientation (score)†	Root morphology (score)‡	Whole plant transpiration ($g\ H_2O\ 24h^{-1}$) §	Water use efficiency ($g\ H_2O\ 24h^{-1}$ g seed yield^{-1})	Single plant seed yield (g plant^{-1})	Plot yield ‡‡ (kg ha^{-1})	Maturity (DAP)	Plant height (cm)	Lodging (score)‡	Seed size (g 100 seed^{-1})	Seed protein (%)††	Seed oil (%) ††	2006 Dry weight (g plant^{-1})	2006 Leaf area (cm^2 plant^{-1})
2 #	60115 - 2	Higher	2.4 *	1.5	909 *	23.1	39.6 *	2018	159	66	2.7	14.6	42.8	18.3	83.4	4279
	60115 - 8	Lower	3.2 *	2.5	581 *	22.8	29.6 *	2013	157	67	3.0	14.6	42.5	18.9	74.9	3727
5	60259 - 10	Higher	2.4 *	2.5	446	17.0	28.3	2700	162	71	1.7	16.7	41.6	18.5	71.4	2682
	60259 - 7	Lower	3.5 *	3.2	590	21.1	27.7	2452	163	70	1.7	15.5	42.2	18.2	69.2	2659
6 #	60502 - 10	Higher	2.6 *	1.8 *	497 *	21.7	23.9 *	2613	166	69	2.0 *	14.4 *	42.8	18.2	57.8	2489
	60502 - 2	Lower	3.6 *	3.0 *	704 *	23.6	30.8 *	2236	168	76	3.2 *	15.8 *	42.1	18.7	63.5	2646
9 #	60558 - 9	Higher	2.6 *	1.8	566	20.2	27.6	2170	166	71	2.0	14.3 *	43.2	18.5 *	78.3	3699
	60558 - 6	Lower	3.7 *	2.7	663	22.0	31.0	2370	164	66	2.2	16.0 *	43.2	17.5 *	93.6	3816
11	70094 - 9	Higher	2.7 *	1.7 *	494	22.8	24.6	1980	164	76	2.2	16.4 *	44.1	18.1	65.8	2515
	70094 - 10	Lower	3.9 *	2.8 *	533	18.5	30.3	2176	163	75	2.5	18.2 *	44.0	18.0	82.8	3242
13	70139 - 8	Higher	2.7 *	2.5	385	19.0	23.7	2726	162	75	2.0	14.0	42.7	18.8	64.1	3417
	70139 - 10	Lower	3.5 *	2.5	317	15.8	23.7	2815	162	75	2.0	14.7	42.5	18.9	69.2	3275
17	70297 - 9	Higher	2.2 *	1.8	574	17.5	27.8 *	2722	154	70	2.0	14.7	42.3 *	19.9	39.7	2654
	70297 - 10	Lower	3.3 *	2.3	781	14.6	40.4 *	2737	157	61	2.2	14.3	41.0 *	19.9	24.4	1776
18 #	70471 - 6	Higher	2.3 *	2.2	522	17.8	30.3 *	2494	167	69	1.7	14.7	41.9 *	18.6	74.3	3337
	70471 - 7	Lower	3.0 *	3.2	385	18.7	22.0 *	2719	167	66	2.0	15.0	43.0 *	18.3	64.1	2823
21 #	70624 - 3	Higher	2.6 *	2.8	441	19.0	26.9 *	2128	163	67	2.0	15.2	43.1	17.7	65.8	3319
	70624 - 6	Lower	3.4 *	1.8	322	20.3	17.4 *	2064	164	66	2.5	14.4	43.2	17.9	65.2	3299
23	70685 - 4	Higher	2.3 *	3.0 *	451	20.7	20.7	1898	141	57	1.5	14.2 *	44.9	18.6	54.5	3113
	70685 - 5	Lower	3.3 *	1.5 *	471	20.8	20.7	2036	141	55	1.5	15.7 *	44.9	18.9	43.1	2084
24 #	70685 - 9	Higher	2.5 *	1.8	511	21.5	22.4	2265	141	62	1.5	15.2	43.7	19.1	46.5	2712
	70685 - 8	Lower	3.6 *	2.0	484	16.1	25.0	2387	143	61	1.7	14.1	43.7	18.9	44.8	2499
25	70821 - 9	Higher	2.6 *	2.7	690	23.1	30.9	2393	166	71	2.2	16.3	41.9	19.3	76.6	2723
	70821 - 6	Lower	3.4 *	3.0	611	24.4	26.2	2574	166	71	2.2	15.7	42.8	19.0	73.1	2512
	$Pr>F_{.05}$		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0025	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal

‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots

§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September. 2006; and 8 August and 7 September, 2007 at Knoxville, TN.

= these lines are common to both the set of 12 line pairs near-isogenic for leaflet orientation and the set of 15 line pairs near-isogenic for root morphology.

†† = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

‡‡ = plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

DAP = days after planting.

* = denotes LSD mean separation difference at the $\alpha = 0.05$ level of significance between the two near-isogenic pair lines.

Table 3.3. Comparison of whole plant transpiration, water use efficiency, single plant yield and plot yield of 12 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of higher and lower leaflet orientation evaluated in 2006 and 2007 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation 2006	2007	2 yr	Root morphology 2006	2007	2 yr	Whole plant transpiration 2006	2007	2 yr	Water use efficiency 2006	2007	2 yr	Single plant seed yield 2006	2007	2 yr	Plot yield # 2006	2007	2 yr
			(score)†			(score)‡			($g\ H_20\ 24h^{-1}$) §			($g\ H_2O\ 24h^{-1}\ g\ seed\ yield^{-1}$)			($g\ plant^{-1}$)			($kg\ ha^{-1}$)	
Higher	12	2.3 b ¶	2.6 b	2.5 b	2.3 a	2.1 a	2.2 a	533 a	558 a	541 a	18.2 a	22.6 a	20.3 a	28.9 a	24.9 a	27.2 a	2649 a	1869 a	2342 a
Lower	12	3.3 a	3.6 a	3.5 a	2.5 a	2.6 a	2.5 a	475 a	586 a	537 a	16.7 a	23.5 a	19.9 a	27.7 a	25.4 a	27.1 a	2650 a	1901 a	2381 a
$Pr>F_{.05}$		<0.0001	<0.0001	<0.0001	0.2552	0.1719	0.1038	0.2656	0.7364	0.9509	0.3408	0.6917	0.7271	0.5471	0.8643	0.9413	0.9952	0.7780	0.7484

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September. 2006; and 8 August and 7 September, 2007.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
# = yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 3.4. Comparison of maturity, height, lodging, seed size, seed protein and oil of 12 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of higher and lower leaflet orientation evaluated in 2005 and 2006 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation 2006	2007	2 yr	Maturity 2006	2007	2 yr	Plant height 2006	2007	2 yr	Lodging 2006	2007	2 yr	Seed size 2006	2007	2 yr	Seed protein 2006	2007	2 yr	Seed oil 2006	2007	2 yr
			(score)†			(DAP)			(cm)			(score)‡			($g\ 100\ seed^{-1}$)			(%)††			(%)††	
Higher	12	2.3 b ¶	2.6 b	2.5 b	157 a	161 a	159 a	72 a	65 a	69 a	2.5 a	1.5 a	2.0 a	16.9 a	13.1 a	15.0 a	41.7 a	44.3 a	42.9 a	18.7 a	18.5 a	18.6 a
Lower	12	3.3 a	3.6 a	3.5 a	157 a	161 a	159 a	70 a	64 a	67 a	2.9 a	1.6 a	2.3 a	17.1 a	13.4 a	15.3 a	41.8 a	44.3 a	42.9 a	18.7 a	18.4 a	18.6 a
$Pr>F_{.05}$		<0.0001	<0.0001	<0.0001	1.0000	0.8825	0.9382	0.6253	0.6893	0.6022	0.1131	0.4880	0.1424	0.6514	0.4675	0.5138	0.8273	0.9741	0.9543	0.8779	0.6238	0.8020

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
†† = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
DAP = days after planting.

Table 3.5. Comparison of dry weight, leaf area, leaf transpiration, stomatal conductance, and photosynthesis of 12 F3:6 and F4:7 near isogenic soybean line pairs separated into classes relative classes of higher and lower leaflet orientation evaluated in 2006 at Knoxville, TN.

Leaflet Orientation Class	Number of lines	Leaflet orientation	Dry weight	Leaf area	Full Sun Leaf Transpiration ‡	Full Sun Leaf Stomatal Conductance ‡	Full Sun Leaf Photosynthesis ‡
		(score)†	(g plant^{-1})	(cm^2 plant^{-1})	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(umol m^{-2} s^{-1})
Higher	12	2.3 b §	64.8 a	3078 a	9.9 a	1.08 a	20.8 a
Lower	12	3.3 a	63.9 a	2863 a	9.9 a	1.07 a	21.7 a
$Pr>F_{.05}$		<0.0001	0.8974	0.3802	0.9866	0.9487	0.3877

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = Transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table 3.6. Comparison of root morphology, leaflet orientation, whole plant transpiration, water use efficiency, single plant yield, plot yield, maturity, lodging, seed size, protein, oil, dry weight and leaf area of 15 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of less prolific and more prolific root morphology evaluated in 2006 and 2007 at Knoxville, TN.

Near isogenic line set	Line	Relative Leaflet Orientation	Root morphology	Leaflet orientation	Whole plant transpiration	Water use efficiency	Single plant seed yield	Plot yield ‡‡	Maturity	Plant height	Lodging	Seed size	Seed protein	Seed oil	2006 Dry weight	2006 Leaf area
			(score)‡	(score)†	(g H_20 $24h^{-1}$) §	(g H_2O $24h^{-1}$ g seed yield^{-1})	(g plant^{-1})	(kg ha^{-1})	(DAP)	(cm)	(score)‡	(g 100 seed^{-1})	(%)††	(%)††	(g plant^{-1})	(cm^2 plant^{-1})
2 #	60115 - 2	Less prolific	1.5	2.4 *	909 *	23.1	39.6 *	2018	159	66	2.7	14.6	42.8	18.3	83.4	4279
	60115 - 8	More prolific	2.5	3.2 *	581 *	22.8	29.6 *	2013	157	67	3.0	14.6	42.5	18.9	74.9	3727
6 #	60502 - 10	Less prolific	1.8 *	2.6 *	497 *	21.7	23.9 *	2613	166	69	2.0 *	14.4 *	42.8	18.2	57.8	2489
	60502 - 2	More prolific	3.0 *	3.6 *	704 *	23.6	30.8 *	2236	168	76	3.2 *	15.8 *	42.1	18.7	63.5	2646
8	60537 - 10	Less prolific	2.2	3.1	705	24.8	32.9	2317	167	75	2.7	13.4 *	42.0	18.2	61.3 *	2566
	60537 - 9	More prolific	3.0	3.0	697	20.5	34.9	2458	168	75	2.2	14.9 *	42.4	18.6	90.7 *	3401
9 #	60558 - 9	Less prolific	1.8	2.6 *	566	20.2	27.6	2170	166	71	2.0	14.3 *	43.2	18.5 *	78.3	3699
	60558 - 6	More prolific	2.7	3.7 *	663	22.0	31.0	2370	164	66	2.2	16.0 *	43.2	17.5 *	93.6	3816
10	60613 - 5	Less prolific	2.8	3.4 *	551	18.9 *	26.7	2721	165	63	1.7	14.5	43.4	18.5	59.6	2428
	60613 - 8	More prolific	3.7	2.9 *	647	30.2 *	22.0	2678	166	66	1.7	13.3	44.1	17.8	63.0	2782
14	70175 - 2	Less prolific	2.7	2.2	529	24.2	21.9	2253	170	77	3.5	13.6	42.4	18.5	66.4	3295
	70175 - 7	More prolific	3.3	2.5	510	20.3	25.2	2480	170	79	3.2	14.7	42.5	18.5	76.0	3459
15	70247 - 2	Less prolific	2.8	3.0	742 *	26.9 *	31.8	2652	166	74	3.0	13.6 *	42.9	18.1	78.8	3540
	70247 - 6	More prolific	3.8	2.8	504 *	19.9 *	27.7	2525	166	70	2.7	15.1 *	42.8	18.3	86.8	3936
16	70259 - 5	Less prolific	2.7	3.0 *	455	17.6	27.2	2396	168	77	2.2	17.8	42.3	18.4	78.3	2940
	70259 - 7	More prolific	3.3	2.5 *	578	21.8	27.0	2852	168	80	2.5	17.7	42.1	18.2	91.3	3253
18 #	70471 - 6	Less prolific	2.2	2.3 *	522	17.8	30.3 *	2494	167	69	1.7	14.7	41.9 *	18.6	74.3	3337
	70471 - 7	More prolific	3.2	3.0 *	385	18.7	22.0 *	2719	167	66	2.0	15.0	43.0 *	18.3	64.1	2823
19	70500 - 5	Less prolific	2.7	3.0	453	17.6	28.2	2428	168	75	2.2	14.8	43.2	18.4	64.1	2405
	70500 - 2	More prolific	3.2	3.1	559	19.3	30.2	2574	168	79	2.2	14.4	43.4	18.1	80.0	3277
20	70558 - 2	Less prolific	1.7	3.5	388	17.5	23.6	2341	167	69	2.5	15.9	43.1	18.0	72.6	3709
	70558 - 8	More prolific	2.3	3.2	388	18.1	21.3	2031	167	66	1.7	16.6	42.9	18.1	69.7	3895
21 #	70624 - 6	Less prolific	1.8	3.4 *	322	20.3	17.4 *	2064	164	66	2.5	14.4	43.2	17.9	65.2	3299
	70624 - 3	More prolific	2.8	2.6 *	441	19.0	26.9 *	2128	163	67	2.0	15.2	43.1	17.7	65.8	3319
22	70638 - 6	Less prolific	1.8	2.9	663	19.3	29.7	2198	155	57	1.5	18.4	41.9	19.3	40.3	2868
	70638 - 2	More prolific	2.7	3.0	568	13.8	32.7	2684	155	62	2.0	17.9	41.8	19.3	31.2	2655
23 #	70685 - 5	Less prolific	1.5 *	3.3 *	471	20.8	20.7	2036	141	55	1.5	15.7 *	44.9	18.9	43.1	2084
	70685 - 4	More prolific	3.0 *	2.3 *	451	20.7	20.7	1898	141	57	1.5	14.2 *	44.9	18.6	54.5	3113
26	70934 - 6	Less prolific	1.5	2.7 *	805 *	35.6 *	24.0	2288	137	65	2.0	14.1 *	42.3	19.9	55.6	2998
	70934 - 5	More prolific	2.2	2.3 *	534 *	24.1 *	20.1	2320	136	67	2.2	15.6 *	42.7	19.1	60.1	2898
	Pr>F $_{.05}$		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0025	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September. 2006; and 8 August and 7 September, 2007.
# = these lines are common to both the set of 12 line pairs near-isogenic for leaflet orientation and the set of 15 line pairs near-isogenic for root morphology.
†† = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
‡‡ = plot yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
DAP = days after planting.
* = denotes mean separation difference at the α = 0.05 level of significance between the two near-isogenic pair lines.

Table 3.7. Comparison of whole plant transpiration, water use efficiency, single plant seed yield and plot yield of 15 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of less prolific and more prolific root morphology evaluated in 2006 and 2007 at Knoxville, TN.

Root Morphology Class	Number of lines	Leaflet orientation			Root morphology			Whole plant transpiration			Water use efficiency			Single plant seed yield			Plot yield #		
		2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr
			(score)†			(score)‡			($g\ H_20\ 24h^{-1}$) §			($g\ H_2O\ 24h^{-1}\ g\ seed\ yield^{-1}$)			($g\ plant^{-1}$)			($kg\ ha^{-1}$)	
Less Prolific	15	2.8 a ¶	3.0 a	2.9 a	2.3 b	1.9 b	2.1 b	510 a	636 a	572 a	17.6 a	25.9 a	21.8 a	28.2 a	25.6 a	27.0 a	2559 a	1924 a	2333 a
More Prolific	15	2.8 a	3.0 a	2.9 a	2.9 a	3.0 a	3.0 a	583 a	518 a	548 a	19.5 a	22.9 a	21.0 a	29.2 a	24.1 a	26.8 a	2637 a	1954 a	2398 a
$Pr>F_{.05}$		0.9036	0.9504	0.9057	0.0182	<0.0001	<0.0001	0.1186	0.1114	0.6266	0.1379	0.3029	0.6271	0.6109	0.4442	0.9053	0.5247	0.7548	0.4920

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal.
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September. 2006; and 8 August and 7 September, 2007.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
# = yield from Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)

Table 3.8. Comparison of maturity, height, lodging, seed size, seed protein and oil of 15 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of less prolific and more prolific root morphology evaluated in 2006 and 2007 at Knoxville, TN.

Root Morphology Class	Number of lines	Root morphology			Maturity			Plant height			Lodging			Seed size			Seed protein			Seed oil		
		2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr	2006	2007	2 yr
			(score)†			(DAP)			(cm)			(score)‡			($g\ 100\ seed^{-1}$)			(%)§			(%)§	
Less Prolific	15	2.3 b ¶	1.9 b	2.1 b	160 a	163 a	162 a	72 a	65 a	69 a	2.7 a	1.8 a	2.3 a	16.7 a	13.1 a	14.9 a	41.7 a	44.1 a	42.8 a	18.5 a	18.5 a	18.5 a
More Prolific	15	2.9 a	3.0 a	3.0 a	160 a	163 a	162 a	73 a	66 a	70 a	2.9 a	1.7 a	2.3 a	16.9 a	13.9 a	15.4 a	41.8 a	44.1 a	42.9 a	18.3 a	18.4 a	18.4 a
$Pr>F_{.05}$		0.0182	<0.0001	<0.0001	1.0000	0.9701	0.9858	0.8112	0.5857	0.6583	0.4073	0.6551	0.8068	0.7739	0.1351	0.3539	0.7003	0.8612	0.7763	0.5366	0.6736	0.5309

† = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
‡ = Lodging = 1 to 5 scale; where 1 = 95% of plants erect; 2.5 = ~50% of plants leaning at angle ≥ 45°; 5 = 95+% of plants leaning at an angle ≥ 45°.
§ = protein and oil reported on a dry weight basis from plot seed at Knoxville, Springfield, Spring Hill, and Milan, TN (all other traits measured at Knoxville, TN)
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.
DAP = days after planting.

Table 3.9. Comparison of dry weight, leaf area, leaf transpiration, stomatal conductance, and photosynthesis of 15 F3:6 and F4:7 near isogenic soybean line pairs separated into relative classes of less prolific and more prolific root morphology evaluated in 2006 at Knoxville, TN.

Root Morphology Class	Number of lines	Root morphology	Dry weight	Leaf area	Full Sun Leaf Transpiration ‡	Full Sun Leaf Stomatal Conductance ‡	Full Sun Leaf Photosynthesis ‡
		(score)†	(g plant^{-1})	(cm^{2} plant^{-1})	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(umol m^{-2} s^{-1})
Less Prolific	15	2.3 b §	65.2 a	3062 a	9.3 a	1.0 a	21.5 a
More Prolific	15	2.9 a	70.9 a	3267 a	9.3 a	0.9 a	21.7 a
Pr>F $_{.05}$		0.0182	0.2974	0.3015	0.9229	0.8048	0.8418

† = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots.
‡ = Transpiration, stomatal conductance, and photosynthesis data obtained from upper canopy leaves exposed to full sunlight.
§ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

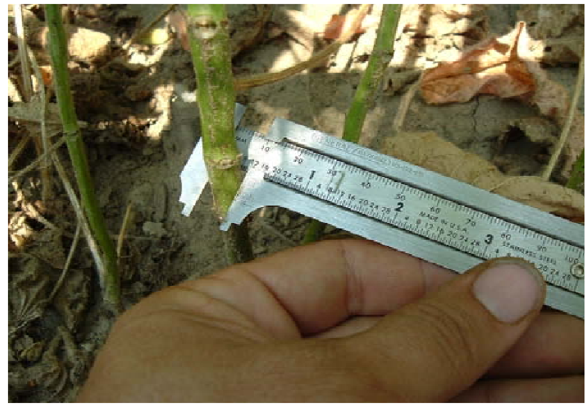
USG 5601 T PI 416937 1.5 score 4.5 score

Figure 3.1. Differences in leaflet orientation at different times of day. Leaflet orientation score is a phenotypic rating on a scale of 1 to 5 with a score of 1 being the condition that the upper canopy leaves were strongly oriented in a paraheliotropic manner with leaflets maintaining a 90º angle to the horizontal plane; 2.5 being leaflets maintaining a 45º angle to the horizontal plane; and 5 being leaflets maintaining an angle parallel to the horizontal plane

USG 5601T PI 416937

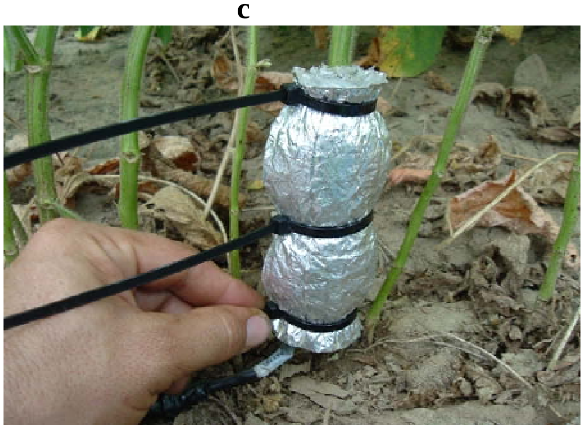
Figure 3.2. Visual rating scale used in scoring root morphology. Root morphology score is a phenotypic rating on a scale of 1 to 5 with 1 being the condition of the plant possessing a normal tap root with few lateral roots and 5 being the condition of the plant possessing a prolific root mass with many fibrous-like lateral branching roots.


a


b


c


d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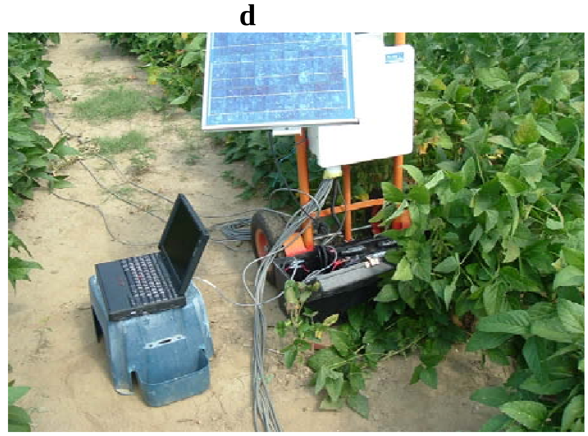
f

Figure 3.3. Use of Dynamax Flow32 System (fitting Dynagauges to soybean stem): a) each plant marked with durable tag for later identification, b) stem diameter measured and recorded, c) stem cleaned of dirt and debris, d) Dynagauge sensor placed around stem with top and bottom sealed with adhesive putty to prevent water and insect infiltration, e) insulating bubble wrap foil placed around Dynagauge (3 layers) and held in place with cable ties securely but with only light pressure, f) part of the Dynamax Flow 32 Sap Monitoring System setup as used in the field experiment showing the attachment to an upright cart for greater mobility, deep cycle marine battery and data link cable inside tool box at bottom, and portable computer for uploading program parameters and collecting data.

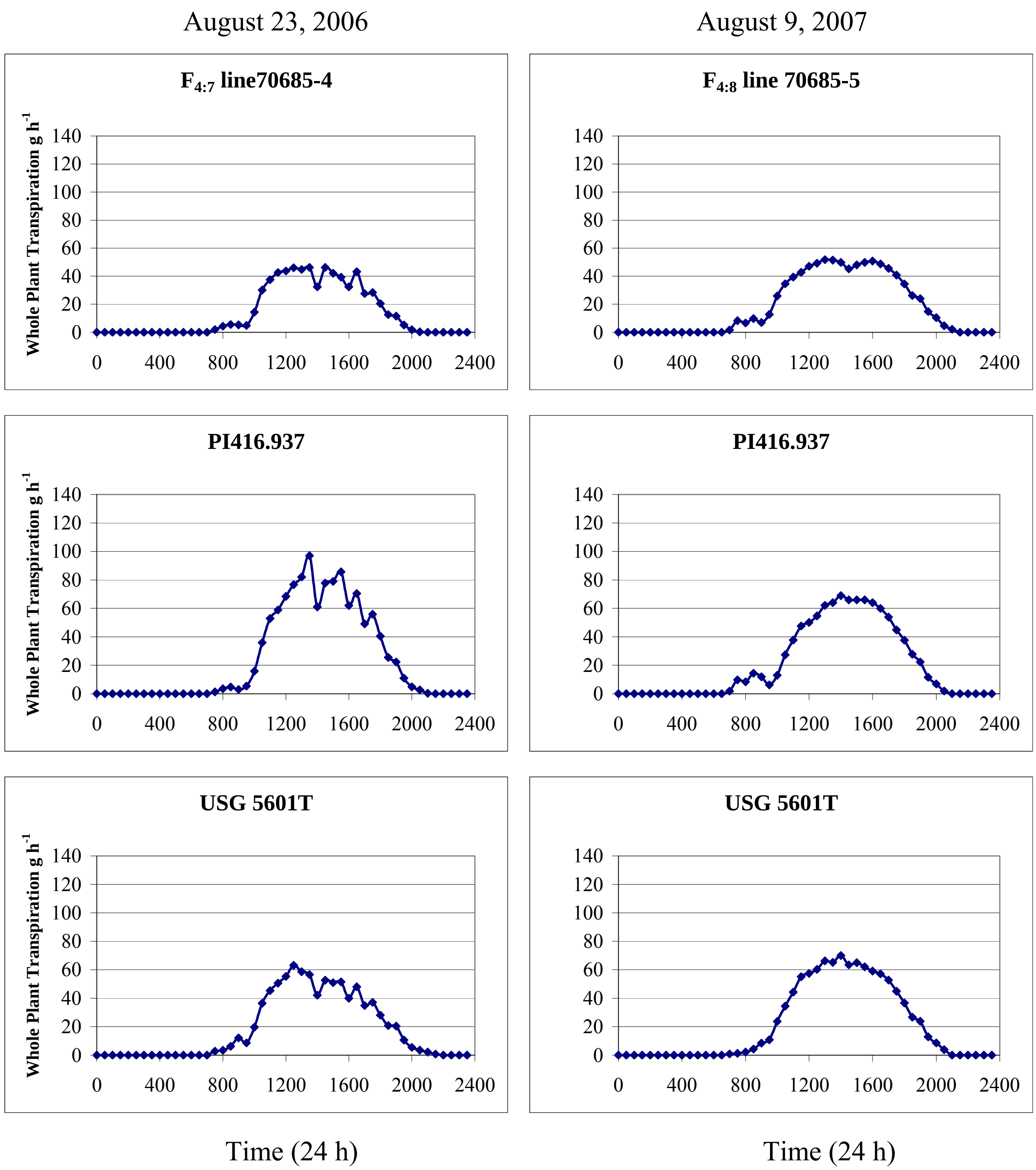

Figure 3.4. Whole plant transpiration measurements of two near-isogenic lines and parental lines recorded during two different 24 hour periods over the two year period, 2006 and 2007. Although transpiration curves displayed are from different measurement days, each is representative of the total average flow for the line in the respective year. The selected lines are represented in each of the measurement days noted in this figure in order to demonstrate similarities in the whole plant transpiration curves across different lines within a given day. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR leaflet temperatures, and transpiration.

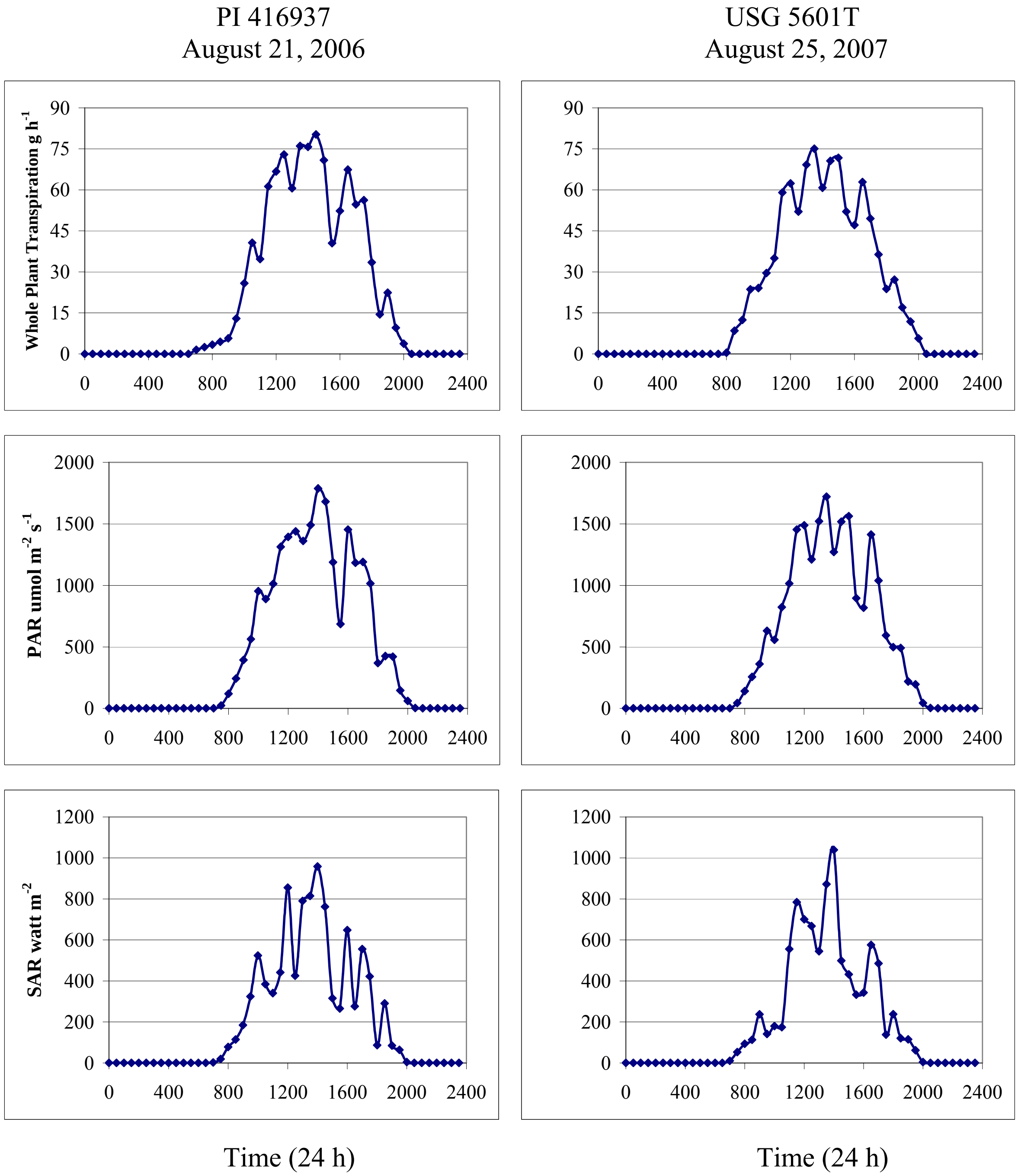

Figure 3.5. Whole plant transpiration, photosynthetically active radiation (PAR) and solar radiation (SAR) measurements of parental lines recorded during two different 24 hour periods in 2006 and 2007. The similarity in the curves within each day demonstrates the close relationship between PAR and transpiration. The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration.

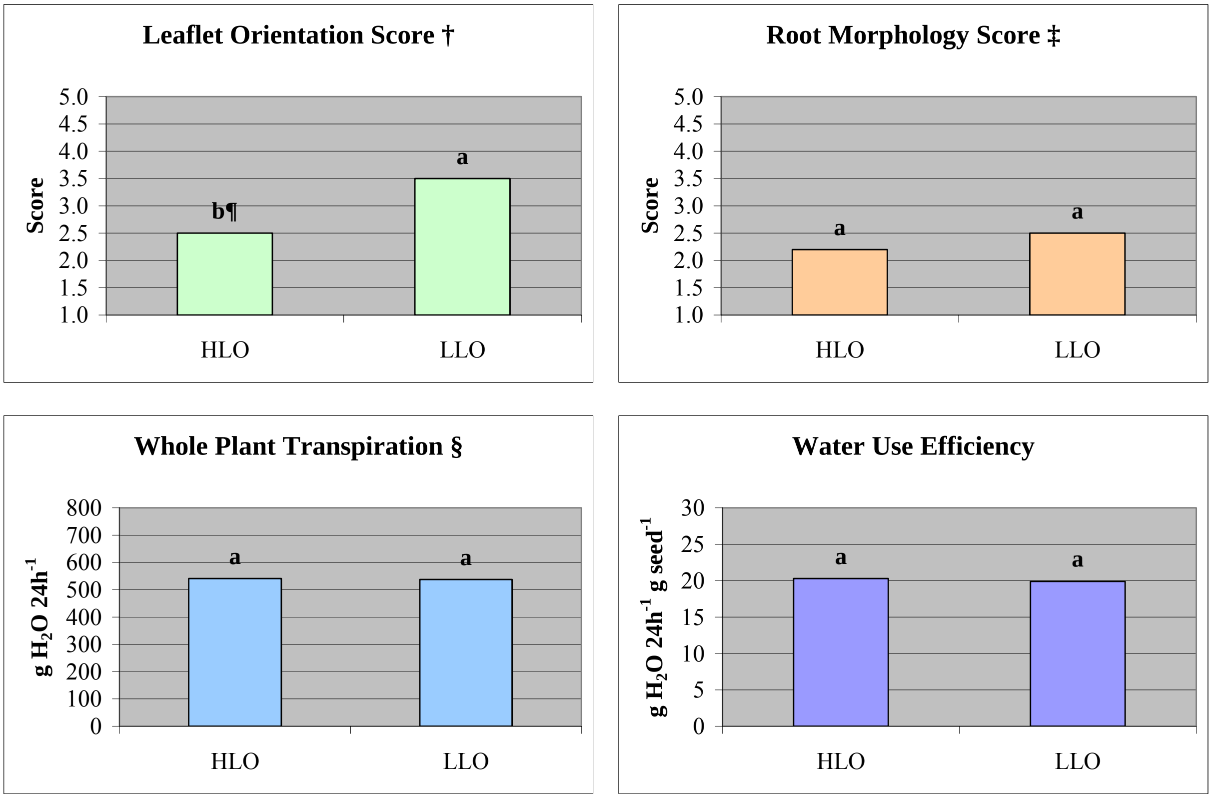

Figure 3.6. Comparison of mean leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 12 F3:7 and F4:8 near isogenic soybean line pairs separated into relative classes of higher (HLO) and lower leaflet orientation (LLO) evaluated for two years, 2006-2007, at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September, 2006; and 8 August and 7 September, 2007.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

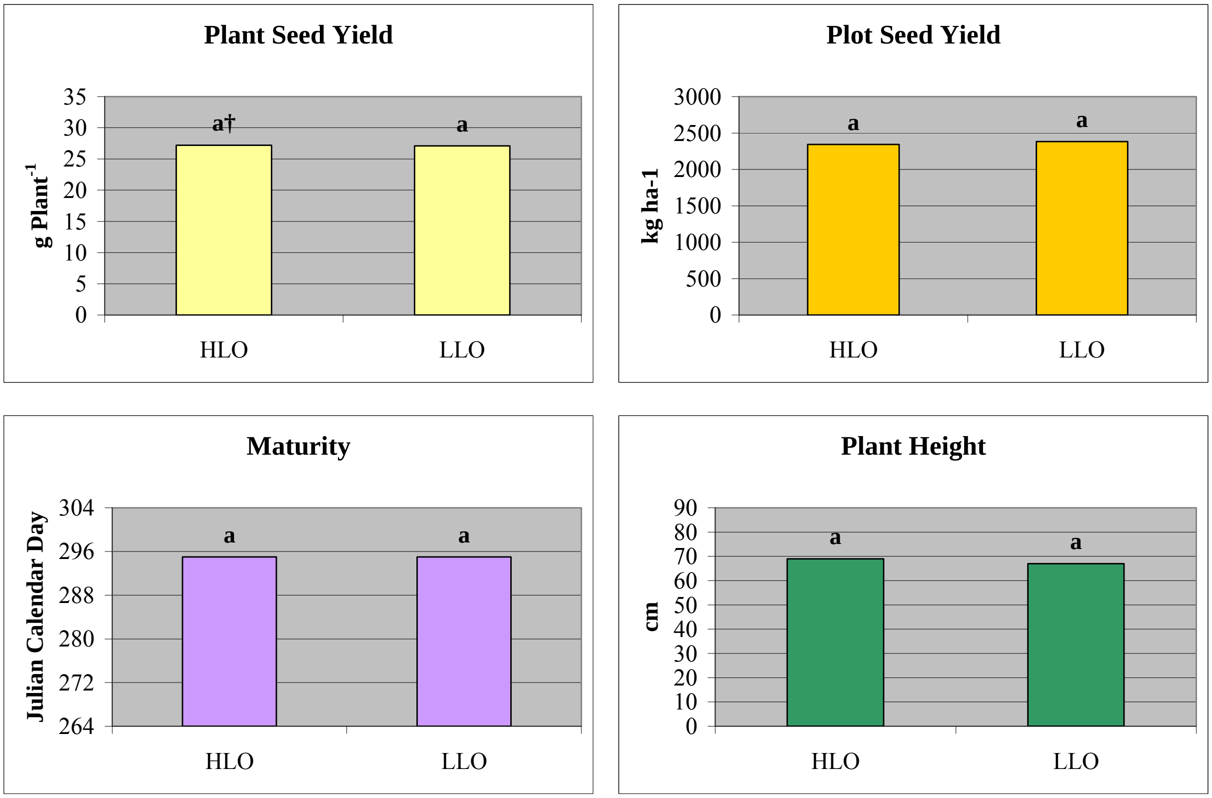

Figure 3.7. Comparison of mean single plant seed yield, plot seed yield, maturity and plant height of 12 F3:7 and F4:8 near isogenic soybean line pairs separated into classes of higher (HLO) and lower leaflet orientation (LLO) evaluated for two years, 2006-2007 at Knoxville, TN.
† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

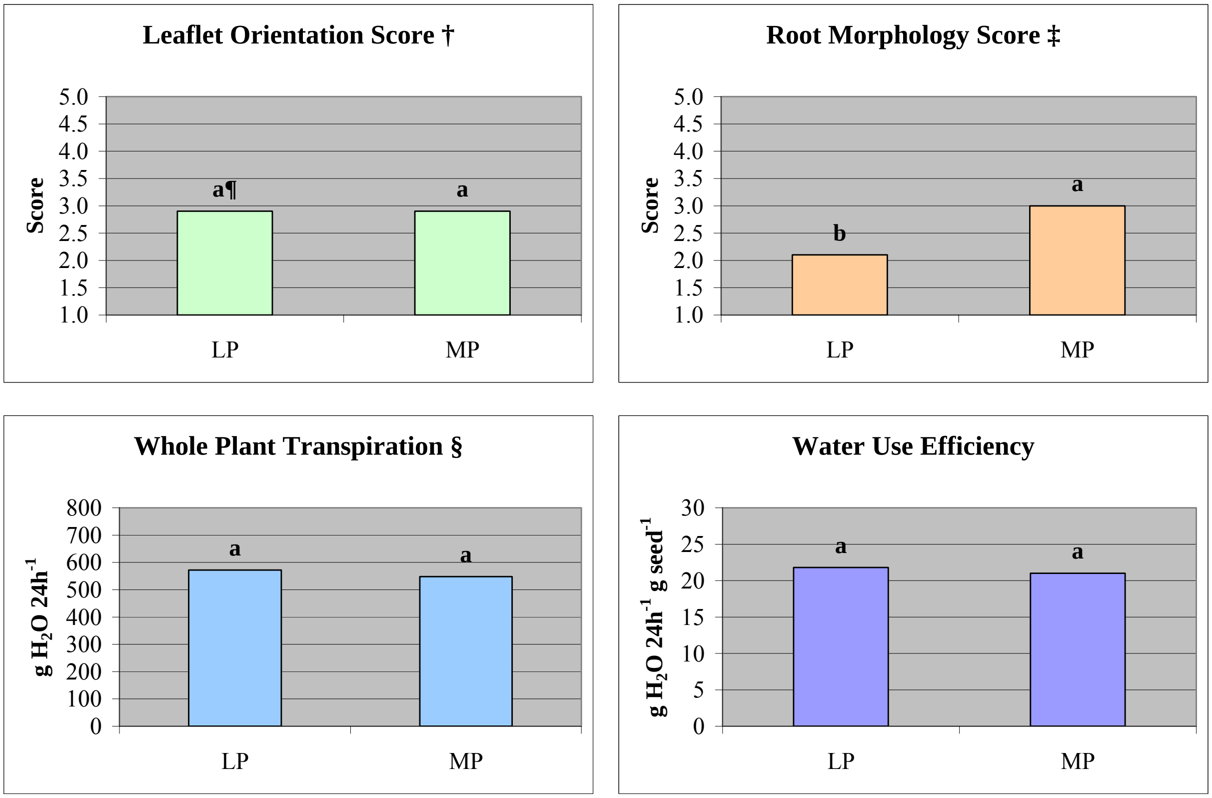

Figure 3.8. Comparison of mean leaflet orientation, root morphology, whole plant transpiration, and water use efficiency of 15 F3:7 and F4:8 near isogenic soybean line pairs separated into classes of less prolific (LP) and more prolific (MP) root morphology evaluated for two years, 2006-2007 at Knoxville, TN.

† = 1 to 5; 1 = leaflets ~ 90º angle to the horizontal plane; 2.5 = leaflets ~ 45º angle to the horizontal plane; 5 = leaflets horizontal
‡ = 1 to 5 scale; 1 = normal tap root with few lateral roots; 5 = prolific root with many fibrous-like lateral branching roots
§ = measurements taken on four plants per line at R4 - R6 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 19 August and 21 September, 2006; and 8 August and 7 September, 2007.
¶ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

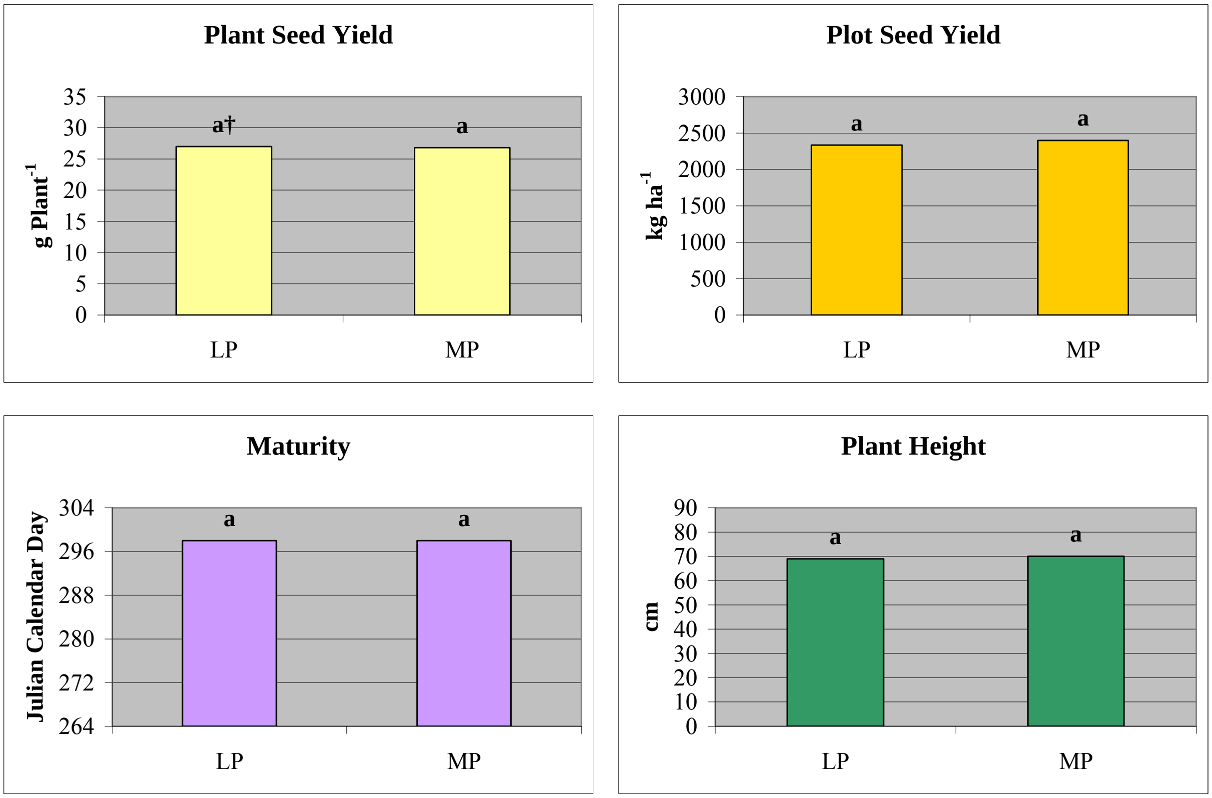

Figure 3.9. Comparison of plant seed yield, plot seed yield, maturity and plant height of 15 F3:7 and F4:8 near isogenic soybean line pairs separated into classes of less prolific and more prolific root morphology evaluated for two years, 2006-2007 at Knoxville, TN.

† = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

Part IV

Effects of Restrained Canopy Leaflet Orientation on Transpiration Rate, Leaflet Temperature and Mid-Canopy Sunlight Penetration in Soybeans

Abstract

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress as it reduces global soybean yield by approximately 40%. Paraheliotropic leaflet movements have been shown to reduce leaflet temperatures and transpiration rates. Soybeans differ in their leaflet orientation response to sunlight. Some soybean genotypes angle their upper canopy leaves so as to avoid direct sunlight at mid-day. Other genotypes remain relatively unchanged throughout the day and do not avoid direct sunlight. The objective of this study was to evaluate the effects of natural and artificially imposed leaflet orientation on transpiration rates and other physiological traits in soybeans. The soybean cultivar USG 5601T was chosen for this study due to its ability to strongly orient its leaves during the day in response to sunlight. Twenty-four plants were subjected to two treatments during the 2007 and 2008 growing season in Knoxville, TN. One treatment set was restrained with netting in order to gently force the orientation of the outer canopy leaves to assume the phenotype of a plant which does not orient its leaves. The other treatment was unrestrained and allowed to orient its leaves as normal. Whole plant transpiration rates of 24 plants for each treatment were measured for a 24 h period with the Dynamax Flow 32 Sap Flow instrument when the plants were in the R5 growth stage of active pod filling. Leaflet temperatures were measured with a Raytek Infrared Thermometer. Photosynthetically Active Radiation (PAR) measurements above and mid-canopy were taken with a Decagon Sunflec Ceptometer. There were no statistical differences found between the average transpiration rates of unrestrained plants, which oriented their leaves and restrained plants which were not allowed to orient their leaves. Temperatures of leaflets which were restrained from movement were

3.3°C higher in temperature than leaflets which were allowed to orient in a paraheliotropic manner. Plants which were unrestrained had significantly higher levels of PAR in their mid-canopy area than plants which were restrained. The high leaflet orientation and associated lower leaflet temperatures of the unrestrained plants may have resulted in a lower transpiration rate for upper canopy leaflets as other studies have found previously. However, the unrestrained soybean plants with high leaflet orientation allowed more sunlight into the lower canopy which may have resulted in higher rates of transpiration and photosynthesis for those leaves relative to the restrained plants with lower leaflet orientation. This may account for the consistent but non-significant 24.4 g d^{-1} increase in whole plant transpiration by the higher leaflet orienting unrestrained plants.

Chapter I

Introduction

Inadequate moisture during flowering and seed-fill is a yield-limiting factor to soybean production throughout many soybean growing regions of the world. Drought is considered the single most important abiotic stress that adversely affects soybean yield by approximately 40% (Pathan et al., 2007). Consequently, drought tolerance is a highly sought after trait in soybean cultivars. Drought tolerance is a complex response and is conditioned by the interaction of several genetic traits of the plant to environmental conditions (Chaves et al., 2003). Knowledge of these trait processes is needed not only for understanding plant resistance to drought stress but also to improve crop management and breeding techniques.

Many species of plants are capable of leaf movements in response to external stimuli (Ehleringer and Forseth, 1980). Leaf movement in response to light, known as heliotropism, can be classified as either diaheliotropic (light seeking) or paraheliotropic (light avoiding). Plants exhibiting diaheliotropism orient the plane of the leaf blade perpendicular to incident light rays, while plants exhibiting paraheliotropism orient the plane of the leaf blade parallel to incident light rays. Soybean exhibits both diaheliotropic and paraheliotropic movements, with the degree of movement being dependent on genotypic response (Wofford and Allen, 1982) and various levels of environmental stimuli (Ehleringer and Forseth, 1989; Rosa and Forseth, 1995).

Paraheliotropism and diaheliotropism provide a means by which the plant can alter the arrangement of its leaves in order to gain maximum benefit from the environment. Advantages of changing leaf angle and light absorbance include increased total canopy light interception (Kawashima, 1969 a,b ; Wang et al., 1994), increased photosynthetic efficiency (Prichard and

Forseth, 1988 He et al., 1996), and increased yield (Wang et al., 1995; Pendleton et al., 1968). Leaflet orientation can also reduce leaf temperature (Forseth and Teramura, 1986; Isoda and Wang, 2002) which can reduce excessive transpiration rates (Bielenberg et al., 2003; Berg and Heuchelin, 1990). Additionally paraheliotropism can reduce photoinhibition (Hirata et al., 1983; Jiang et al., 2006), and increase water use efficiencies (Rosa et al., 1991; Kao and Tsai, 1998). In soybean, this phenomenon may be a mechanism to reduce water loss while maintaining some level of productivity as reported by Meyer and Walker (1981). Paraheliotropic leaf movements reduce transpirational water loss by lowering light interception of leaves, consequently improving water status and lowering leaf temperature. In soybean, this phenomenon may be a mechanism to reduce water loss while maintaining some level of productivity as reported by Meyer and Walker (1981). Paraheliotropic leaf movements reduce transpirational water loss by lowering light interception of leaves, consequently improving water status and lowering leaf temperature.

Research conducted at the University of Tennessee (Wofford and Allen, 1982) demonstrated that soybean cultivars differ in their ability to orient leaflets during the course of the day. Most cultivars exhibit high leaflet orientation (paraheliotropism) and move their leaves during the course of the day such that the leaves have maximum exposure to the sun in the early and late parts of the day, but during mid-day the leaves are oriented parallel to sunlight such that the surface of the leaves has minimum exposure to the sun. A lesser number of cultivars exhibit low leaflet orientation where the leaf surface remains relatively flat and changes little relative to the position and intensity of sunlight, even during the mid-day period of highest irradiance (Fig. 4.1). These “low leaflet orienting” types are therefore relatively less paraheliotropic. In a study of the cultivar Essex (high leaflet orientation) and Dare (low leaflet orientation), the two cultivars produced about equal yields; however Essex used about one-half the amount of water as Dare during the growing season (Paris, 1997).

Kawashima (1969 a,b) found that soybean leaflets exhibiting paraheliotropism in the upper canopy allowed light to penetrate more deeply into the canopy, increasing photosynthetic output of the lower leaves, thus allowing total photosynthetic efficiency of the plant to be improved. Vertical leaf angles decrease the amount of solar radiation intercepted by the leaf. However photosynthetic rate response in plants to solar radiation is nonlinear and saturates below the intensity of direct ambient sunlight (van Zanten et al., 2010). Soybeans are reported to maximize their photosynthetic rates at less than one-third the amount of full sunlight according to Beuerlein and Pendleton (1971).

Grant (1999) found that soybean plants that exhibit paraheliotropism are able to reduce UV-B irradiance in contrast to plants that do not orient leaflets. Ikeda and Matsuda (2002) studied photosynthetic efficiency differences in soybean leaves which were restrained from orienting versus naturally orienting. Their results indicated that paraheliotropic leaflet movements are an adaptation which optimizes net leaflet photosynthesis.

Isoda et al. (1992, 1993) found that the paraheliotropic movements of soybean leaflets regulate light interception and reduce leaf temperature. Isoda and Wang (2002) studied leaf temperature and transpiration rates of cotton versus soybeans and found that soybeans were able to reduce leaf temperatures and transpiration rates. This was attributed to the soybean cultivars ability orient its leaves in a paraheliotropic manner. In a study involving restrained and unrestrained soybean leaflets, Isoda and Tomagae (2003) found differences in temperature of up to 5.5 degrees C between restrained and unrestrained leaflets of the same soybean cultivar.

Isoda and Tomagae (2003) compared biomass and seed yields of a highly orienting soybean cultivar which had its upper canopy leaves restrained from flowering to harvest in contrast to the same unrestricted cultivar. The study detected no differences in biomass or seed yields between the forced “low orienting” treatment and the “high orienting” control. There were also no differences detected in photosynthetic efficiencies or photoinhibition which may have been influenced by genotypic and/or environmental effects noted in the study as the results are contrary to previous research on the photosynthetic and photoprotective advantages of leaflet orientation (Shaw and Weber, 1967; Prichard and Forseth, 1998; Ikeda and Mastuda, 2002; Wang et al., 1995; Jiang et al., 2006; Hirata et al., 1983; Rosa et al., 1991; Rosa and Forseth, 1995; Kao and Tsai, 1998).

The objective of this research was to investigate the effects of leaflet orientation on transpiration, leaflet temperature, and canopy light penetration by comparing these measured triats as exhibited by a high leaflet orienting line which had its upper canopy leaflets restrained verses unrestrained in order to conform to the phenotypic classes of low leaflet orientation and high leaflet orientation, respectively.

Chapter II

Materials and Methods

Experiments were conducted at Knoxville, TN USA (35.89 lat., 83.96 long.) during the 2007 and 2008 growing seasons to evaluate the effects of leaflet orientation on transpiration, leaflet temperature, and canopy light penetration in soybean. In order to reduce differences due to genetic factors other than leaflet morphology, a single, highly homozygous, cultivar with high degree of leaflet orientation (USG 5601T) was used in this study to evaluate the effects of leaflet orientation on the measured traits. USG 5601T is a recently released high yielding, maturity group 5, determinate cultivar which is well adapted to the growing environment (Pantalone et al., 2003).

Plots were seeded using a commercial planter (John Deere, Max Emerge, Moline, IL) equipped with plot cone type seeding units (model CTS, Almaco, Nevada, IA). All plots were seeded at a density of approximately three cm apart into single row plots which were six meters in length with 76.2 cm spacing rows. In 2007 plots were planted on a Staser Silt Loam soil (fine-loamy, mixed, active, thermic, Cumulic Hapludoll). In 2008 plots were planted on an Etowah Silt Loam soil (fine-loamy, siliceous, semiactive, thermic Typic Paleudult).

During the 2007 and 2008 growing seasons, two bordered rows of USG 5601T were grown next to each other. One row was restrained with four inch (10.16 cm) mesh bird netting (U.S. Netting Inc., Erie, PA) in such a way as to gently force the orientation of the outer canopy leaves to assume the phenotype of a plant which does not orient its leaves. The other row was also covered with the same netting; however, the netting was suspended so it did not restrain the leaflets (Fig. 4.2). Whole plant transpiration rates were measured on several successive days using the Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stage (R5). Although this measurement may not be representative of transpiration over the growing season, it is deemed important as it represents the period in which seed yield and seed quality constituents are developed and water use is at or near its peak (Wilson, 2004; Heatherly and Elmore, 2004). Consequently, this is also the approximate period when leaflet orientation values were found to be at their highest by Wofford and Allen (1982). Dynamax model SGA9 Flow32 System Dynagauges were used to connect each plant to the system as the approximate 9mm diameter size of the Dynagauge would properly fit around the lower stem. Each plant was marked with a durable tag for identification purposes later in the season. The stem diameters were measured and cleaned. The interior of the Dynagauge sensor was lubricated with a very thin film of Dow Corning 4 Electrical Insulating Compound (Dow Corning Corp, Midland, MI) and then placed around the stem in such a manner as to ensure that the thermocouples and heater strip of the sensor were in direct contact with the stem. The top and bottom of the sensor was then sealed with Elmer's Poster Tack adhesive putty (Elmer's Products Inc., Westerville, OH). The sensor was then wrapped with a sheet of Reflectix double reflective insulation (Reflectix Inc., Markleville, IN) measuring approximately 14 cm x 33 cm which provided two layers of insulation. The insulation was held in place by placing a cable tie near the top, bottom and middle of the sensor in a manner such that the insulation is secure but with minimal pressure being applied to the stem. The Dynamax Sap Flow32 system was mounted to a vertical cart with wheels for easier transportation within the field. The battery and data cables were placed in a large tool box also mounted to the cart. Additionally a solar panel was attached to the cart to extend the battery life and operating capacity of the system (Fig. 4.3).

Whole plant transpiration data were collected on 12 plants from each canopy treatment over a period of two to four days depending on the environmental conditions. The goal was to collect data from a 24 h period when the conditions were mostly sunny, therefore some measurements covered a longer period of time due to cloudy days after the system was installed on the plant material. Immediately following the initial measurements, the canopy netting treatments were switched from one plot row to the other and additional measurements were taken. Each day represented a replication of the experiment in each year. Days and year were considered random effects in analysis using SAS Proc Mixed. Thus each canopy treatment had a total of 12 observations per replication per year for a total of 24 observations each year and 48 observations over the two year period. Transpiration data (g H_2O $24h^{-1}$) from a single mostly sunny day from each replication of the experiment were used in this analysis. Data collected on other days were not utilized due to factors such as sensor malfunctions and/or environmental conditions. Whole plant transpiration data were collected on 24 and 25 August, 2007 and on 10 and 12 September, 2008.

Photosynthetically active radiation (PAR), solar radiation (SAR), and soil moisture were recorded at the field location using a Hobo® weather station equipped with the H21-001 data logger, a S-LIA-M003 PAR sensor, a S-LIB-M003 pyranometer SAR senor, and S-SMA-M003 soil moisture sensors (Onset computer corporation, Pocasset, MA). Leaflet temperatures were measured with a Raytek model ST20 Pro infrared thermometer (Raytek Corp., Santa Cruz, CA). Four replications of leaflet temperatures were recorded on 26 August, 2007 for each treatment between the hours of 1200 and 1400. PAR measurements above and mid-canopy were taken with a model SF40 Decagon Sunflec Ceptometer (Decagon Devices Inc., Pullman, WA). Four replications of PAR levels were recorded on 12 September, 2008 for each treatment between the hours of 1200 and 1600.

All analyses were performed using SAS Proc Mixed with the leaf restraining treatments considered as fixed effects and the days, replications and years considered as random effects (SAS User Guide 9.1.3, 2006). Least squares means with mean separation and LSD values were obtained using the SAS macro written by Saxton (1998).

Chapter III

Results and Discussion

Transpiration in the soybean plants during the monitoring period appeared to began at approximately 0800 h, reaching a peak at approximately 1500 h, and ceasing at approximately 2000 h. While transpiration curves of restrained and unrestrained plants differed in overall magnitude, they were somewhat similar in shape within a day of measurement. There were variations in the transpiration curves overall shape between days due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR leaflet temperatures, and transpiration (Fig 4.4). The similarities observed between the transpiration curves and the PAR curves within each day demonstrates the close relationship between PAR and transpiration. The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis and transpiration (Fig. 4.5). These observations are similar to those reported previously for soybean by Gerdes et al.(1994).

There were no statistical differences ($p \leq 0.05$) found between the average transpiration rates of unrestrained plants, which oriented their leaves and restrained plants which were not allowed to orient their leaves (Table 4.1, Fig. 4.6). The unrestrained treatment plants of USG5601T transpired an average of 359.2 g H_2O $24h^{-1}$ which is and average of 24.4 g more than the restrained plants (370.8 g H_2O $24h^{-1}$). This was consistent in both years of the test; however the difference is too small to be interpreted as a trend. This is contradictory to previous research which indicates that paraheliotropic leaf movements lower both leaflet temperatures and leaflet transpiration (Prichard and Forseth, 1988; Bielenberg et al., 2003; Kao and Forseth, 1992a; Yu and Berg, 1994; Isoda and Wang, 2001, 2002; Wien and Wallace 1973; Shackel and Hall, 1979; Meyer and Walker, 1981; Berg and Hsiao, 1986; Forseth and Teramura, 1986; Berg and Heuchelin, 1990) The current study did find that the temperatures of leaflets which were unrestrained and allowed to orient in a paraheliotropic manner were 3.3°C lower in temperature than leaflets which were retrained from movement (Fig 4.7). This is similar to results found by many researchers on a somewhat wide variety of plants (Prichard and Forseth, 1988; Gamon and Pearcy, 1989; Forseth and Teramura, 1986; Kao and Forseth, 1992a; Paris, 1997; Yu and Berg, 1994; Rosa and Forseth, 1995; Isoda and Wang, 2002; Arena et al., 2008; He et al., 1996; Stevenson and Shaw, 1971; Isoda and Tomagae, 2003). Plants which were unrestrained had significantly higher levels of PAR in their mid-canopy area than plants which were restrained (Fig. 4.8). Middle canopy PAR measurements were an average of 241 umol m^{-2} s^{-1} compared to only 64 umol m^{-2} s^{-1} for the restrained treatments as the upper leaves prevented sunlight penetration. This represents a 83% reduction in middle canopy PAR levels of the unrestrained, leaflet orienting treatment compared to a 95% reduction in the restrained, non-orienting treatment. These results agree with observations by other researchers which indicate that by orienting upper canopy leaves plants may allow more light to penetrate into the lower canopy (Kawashima, 1969 a,b ;Wein and Wallace, 1973; Wang et al., 1994; Reynolds et al., 2000; Isoda and Wang, 2001). This may increase the overall amount of light interception and photosynthesis of the plant, however it is not known what effect this may have had on photosynthesis as no instrumentation for collecting that data was available at the time of measurement.

Chapter IV

Conclusions

Based on previous studies involving leaflet orientation it was initially hypothesized that plants which orient their leaves to reduce irradiance and lower leaf temperature might also lower overall transpiration rates. The high leaflet orientation and associated lower leaflet temperatures of the unrestrained plants may have resulted in a lower transpiration rate for upper canopy leaflets, as other studies have previously found. However, the unrestrained soybean plants with high leaflet orientation allowed more sunlight into the lower canopy which may have resulted in higher rates of photosynthesis and transpiration for those leaves relative to the restrained plants with lower leaflet orientation. The more open canopy of the unrestrained high leaflet orienting plants may also allow more air flow and decreased humidity which would also tend to increase transpiration rates. These observations may account for the consistent but non-significant 24.4 g d^{-1} increase in transpiration by the higher leaflet orienting unrestrained plants.

Purcell (2006) stated the main tenets of crop physiology is that crop mass and yield are proportional to the cumulative amount of light intercepted and the amount of water transpired by the crop during a season. Research indicates this to be true although the relationships may be more curvilinear than previously perceived (Edwards et al., 2005; Purcell et al., 2007). It may be of importance to note that most commercial lines, which have been extensively selected for high yield, appear to have high leaflet orientation morphology. This was recently observed as part of the authors work with soybean variety testing coinciding with the current experiment. High leaflet orientation may allow plants to achieve increased photosynthesis rates contributing to higher yield, as has been documented in other studies and crops (Wang et al., 1995; Chang and

Tagumpay, 1970; Mickelson et al., 2002; Pendleton et al., 1968; Pepper et al., 1977). While this could lead to an overall increase in the amount of water transpired, it may also be a mechanism which reduces excessive transpiration by upper canopy leaves which would otherwise be irradiated to oversaturation. In this manner the leaflet orientation trait may allow a balance of reducing excessive water loss while allowing a maximization of light interception, photosynthesis and yield. The current study was limited to examining effects of leaflet orientation on whole plant transpiration, leaflet temperature and canopy light penetration. Further study is needed to determine the effects of leaflet orientation on overall photosynthesis rates, transpiration and yield in soybeans.

Acknowledgements

Trade names or commercial products were mentioned solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the University of Tennessee.

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APPENDIX A

Part IV

Table 4.1. Whole plant transpiration rate differences of USG 5601T soybean plants with upper canopies Unrestrained (high leaflet orientation) versus Restrained (low leaflet orientation) over the two year period (2007 - 2008) at Knoxville, TN.

Year	Treatment	Leaflet Orientation Class	Whole plant transpiration †	Transpiration Difference
			($g\ H_20\ 24h^{-1}$)	($g\ H_20\ 24h^{-1}$)
2007	Unrestrained	High Leaflet Orientation	459.9 a ‡	23.8
	Restrained	Low Leaflet Orientation	436.1 a	
2008	Unrestrained	High Leaflet Orientation	331.0 a	25.8
	Restrained	Low Leaflet Orientation	305.3 a	
2 Year	Unrestrained	High Leaflet Orientation	395.2 a	24.4
	Restrained	Low Leaflet Orientation	370.8 a	

† = measurements taken on 12 plants per treatment at R5 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 24 and 25 August 2007; and 10 and 12 September 2008.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD. Pr>F .05 = 0.6947, 0.4581, and 0.4744 in the 2007, 2008 and 2 year data sets, respectively.

High leaflet orienting Low leaflet orienting High leaflet orienting Low leaflet orienting

Figure 4.1. Differences in leaflet orientation at different times of day.

Figure 4.2. Netting placed over the upper canopy leaves of USG 5601T soybean line was used to condition an unrestrained "high leaflet orientation" treatment (left) and restrained "low leaflet orientation" treatment (right). USG 5601T normally exhibits high leaflet orientation when unrestrained. Restrained leaves were gently manipulated to the desired orientation once the netting was secured around the upper plant canopy.

Figure 4.3. Use of Dynamax Flow32 System (fitting Dynagauges to soybean stem): a) each plant marked with durable tag for later identification, b) stem diameter measured and recorded, c) stem cleaned of dirt and debris, d) Dynagauge sensor placed around stem with top and bottom sealed with adhesive putty to prevent water and insect infiltration, e) insulating bubble wrap foil placed around Dynagauge (3 layers) and held in place with cable ties securely but with only light pressure, f) part of the Dynamax Flow 32 Sap Monitoring System setup as used in the field experiment showing the attachment to an upright cart for greater mobility, deep cycle marine battery and data link cable inside tool box at bottom, and portable computer for uploading program parameters and collecting data.

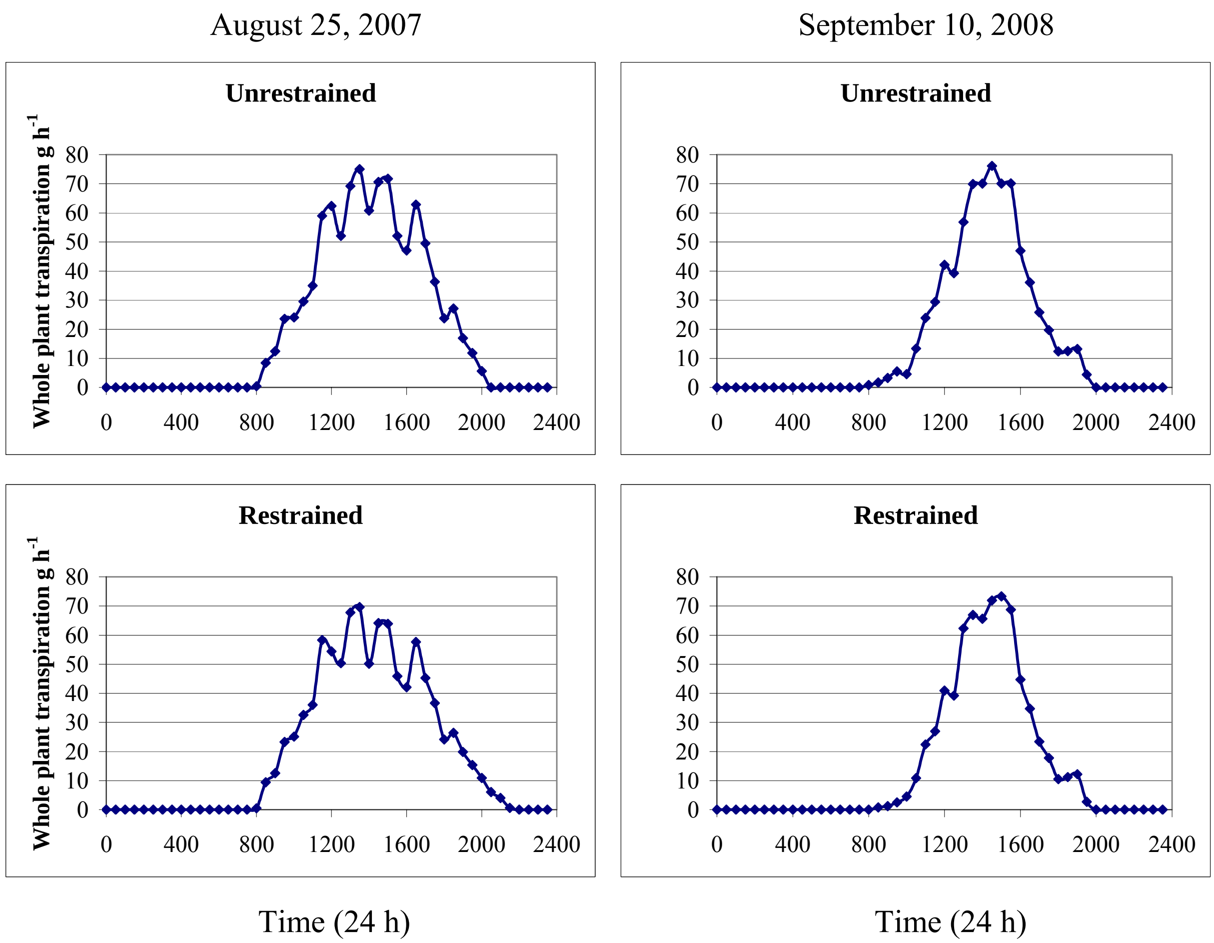

Figure 4.4. Whole plant transpiration measurements of unrestrained and restrained USG 5601T soybean plants recorded during two different 24 hour periods over the two year period, 2007 and 2008 at Knoxville, TN. Plants were unrestricted (high leaflet orientation) or restricted (low leaflet orientation) by netting in order to condition the desired leaflet orientation. Although transpiration curves displayed are from different measurement days, each is representative of the total average flow for the treatment in the respective year. The selected plants are represented in each of the measurement days noted in this figure in order to demonstrate similarities in the whole plant transpiration curves across different treatment plants within a given day. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR leaflet temperatures, and transpiration.

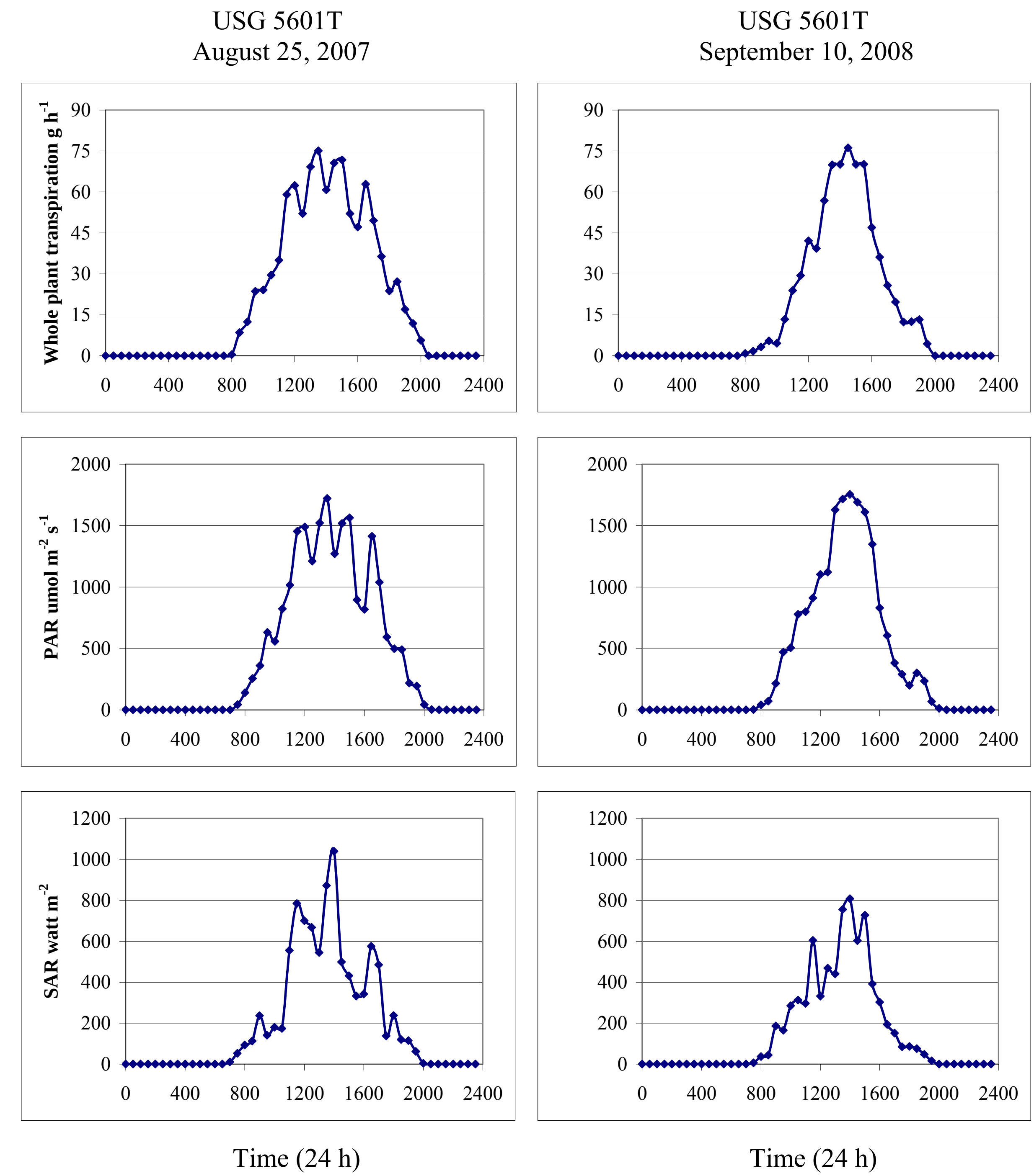

Figure 4.5. Whole plant transpiration, photosynthetically active radiation and solar radiation measurements of unrestrained USG 5601T soybean lines recorded during two different 24 hour periods in 2007 and 2008. The similarity in the curves within each day demonstrates the close relationship between PAR and transpiration. The solar radiation curve, while still being somewhat analogous, is less similar to the transpiration curve as it is a measure of total radiation and includes additional wavelengths which have less importance to photosynthesis. Variations in transpiration curves overall shape between days are due to environmental conditions such as passing cloud cover, which differed by day, and reduced PAR, SAR, leaflet temperatures, and transpiration.

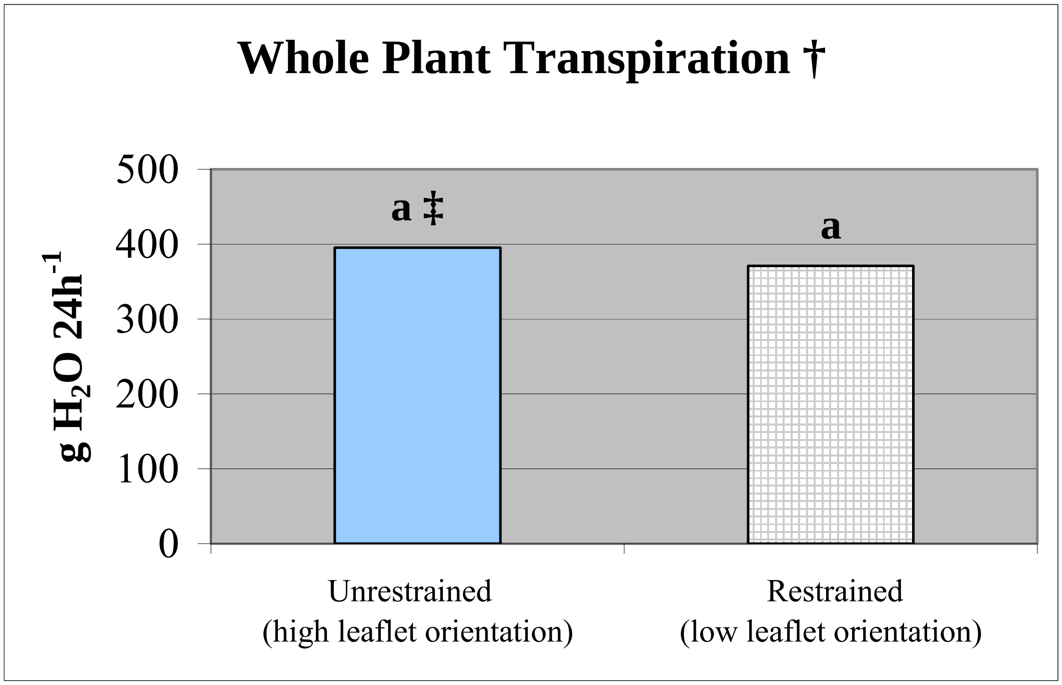

Figure 4.6. Whole plant transpiration rates of USG 5601T soybean plants with upper canopies unrestrained (high leaflet orientation) versus restrained (low leaflet orientation) over the two year period (2007 - 2008).

† = measurements taken on 12 plants per treatment each year at R5 growth stage with Dynamax Flow 32 Sap Flow Monitoring System ™ between the dates of 24 and 25 August 2007; and 10 and 12 September 2008.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

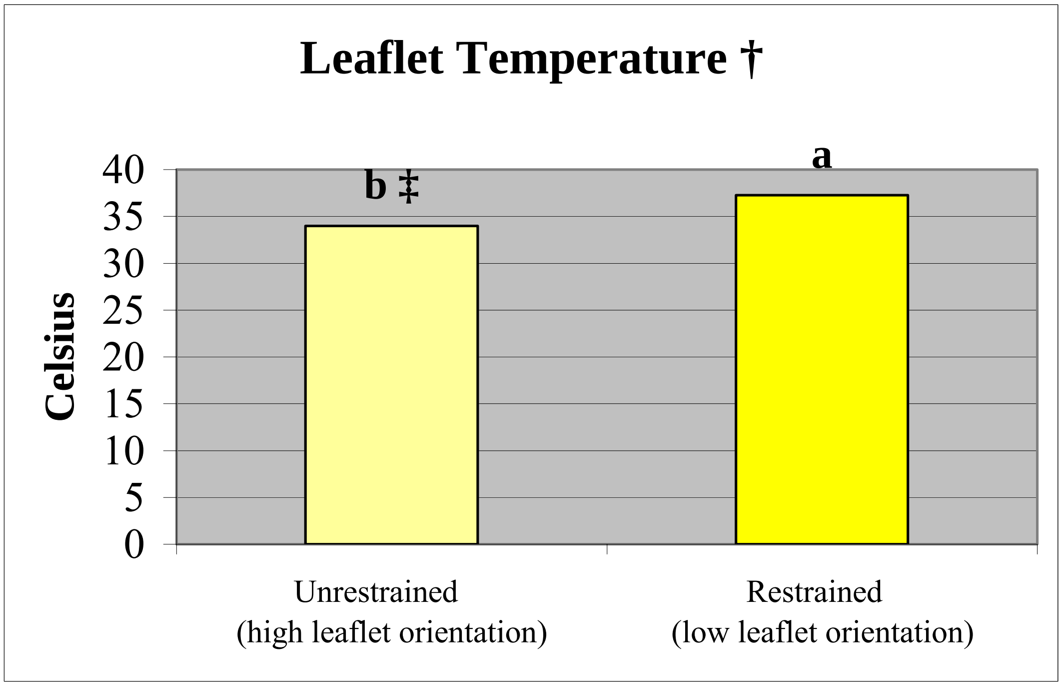

Figure 4.7. Leaflet temperatures of USG 5601T soybean plants with upper canopies unrestrained (high leaflet orientation) versus restrained (low leaflet orientation) at Knoxville, TN in 2007.
† = measurements taken on 4 plants per treatment R5 growth stage with Raytech ST20 Pro infrared thermometer on 26 August 2007.
‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

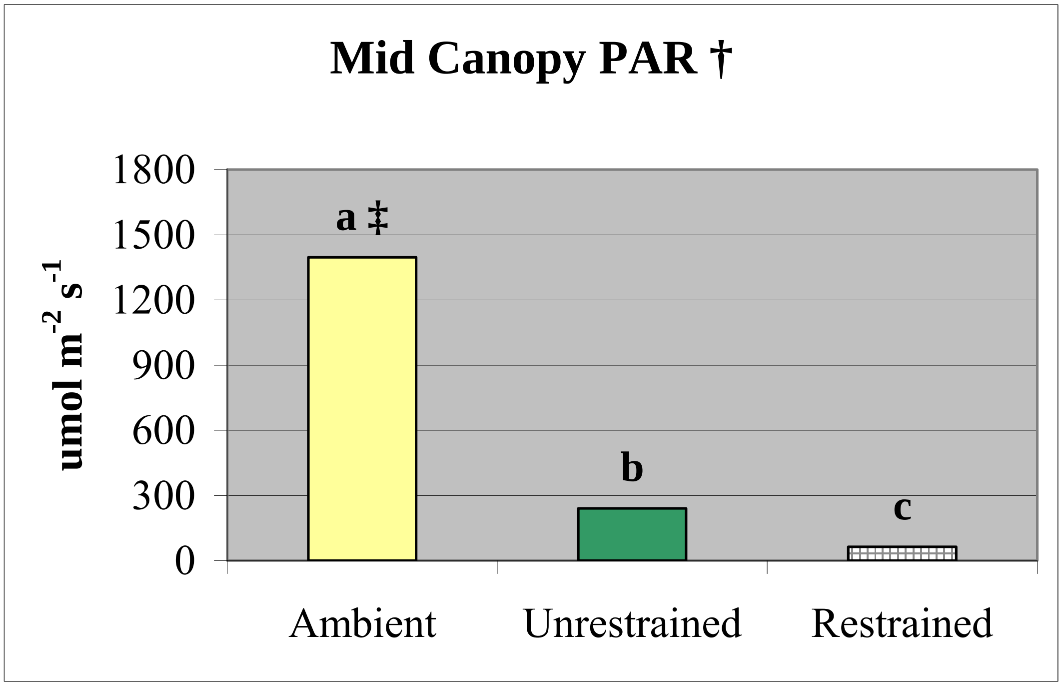

Figure 4.8. Photosynthetically Active Radiation (PAR) levels of ambient (above canopy), unrestrained mid-canopy, restrained mid-canopy treatments of USG 5601T at Knoxville, TN in 2008.

† = PAR for each treatment was measured four times with a Decagon Sunflec Ceptometer on 12 September, 2008 at the hours of 1230, 1400, 1500, and 1620.

‡ = measurements followed by the same letter are not different at the $\alpha = 0.05$ level of significance based on the LSD.

APPENDIX B

Part IV

Supplemental Tables

Table B.4.1. Leaflet temperatures of USG 5601T soybean plants with upper canopies unrestrained (high leaflet orientation) versus restrained (low leaflet orientation) at Knoxville, TN in 2007.

Treatment	Leaflet Orientation Class	Whole plant transpiration †	Transpiration Difference
		°C	°C
Unrestrained	High Leaflet Orientation	33.9 b ‡	3.3
Restrained	Low Leaflet Orientation	37.2 a	

† = measurements taken on 4 plants per treatment R5 growth stage with Raytech ST20 Pro infrared thermometer on 26 August 2007.
‡ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

Table B.4.2 Photosynthetically Active Radiation (PAR) level differences of ambient (above canopy), unrestrained mid-canopy, and restrained mid-canopy treatments of USG 5601T at Knoxville, TN in 2008.

Treatment / Position	PAR †
	(umol m^{-2} s^{-1})
Ambient / above canopy	1396 a ‡
Unrestrained / mid-canopy	241 b
Restrained / mid-canopy	64 c
$Pr>F_{.05}$	< 0.0001

† = PAR for each treatment was measured four times with a Decagon Sunflec Ceptometer on 12 September, 2008 at the hours of 1230, 1400, 1500, and 1620.
‡ = measurements followed by the same letter are not different at the α = 0.05 level of significance based on the LSD.

SUMMARY

Drought is considered the single most important abiotic stress that adversely affects soybean yield. Adaptive traits which condition dehydration avoidance include those which minimize excessive temperature stress and water loss and those which maximize water uptake. Two potential traits of interest are leaflet orientation and root morphology. Leaflet orientation has been shown to reduce leaflet temperatures and water loss while root morphology has been related to slower wilting phenotypes. The objective of this study was to investigate the effects of leaflet orientation and rooting morphology, both singly and in combination, on whole plant transpiration, seed yield, water use efficiency and other physiological and agronomic traits in soybeans. This was accomplished in four parts: grafting experiments, population development and evaluation, near-isogenic line development and restrained canopy leaflet experiments.

Grafting experiments

The grafting experiments were conducted at Knoxville, TN (35.89 lat., 83.96 long.) during the 2003 growing season. Three soybean cultivars were chosen: USG 5601T, PI 416937 and Williams 82 which differed in leaflet orientation, root morphology, and other characteristics. Twelve treatments consisting of non-grafted plants of each cultivar, self grafts and reciprocal grafts of scion and rootstocks were made among the three cultivars. Whole plant transpiration of plants was measured on several successive days via a Dynamax Flow 32 Sap Flow Monitor™ when the plants were in the R4-R6 stage of growth. Data for leaflet orientation, rooting morphology, seed yield, photosynthetically active radiation (PAR), solar radiation (SAR), plant height, seed size, seed protein and oil were also recorded for all treatments. No significant differences were detected between the non-grafted and self grafted treatments of each line for

leaflet orientation, root morphology, whole plant transpiration, water use efficiency, seed size, or plant height. The significant differences detected for seed yield, seed protein and oil between the self grafted and non-grafted treatments of PI 416937 may indicate an effect due to grafting technique, which in this study, appeared to be limited to these three traits of this one line. Leaflet orientation, seed yield, water use efficiency, seed size, plant height, seed protein and oil were all significantly affected by the scion treatment indicating that these traits were conditioned predominately by the shoot portion of the plant. Root morphology scores were not significantly different ($p \leq 0.05$) among scion treatments, indicating that the root morphology is conditioned independently from the upper part of the plant. Whole plant transpiration was not significantly different ($p \leq 0.05$) among scion treatments. Root morphology score was the only trait which was significantly different ($p \leq 0.05$) among the rootstock treatments. None of the other measured traits were significantly different when comparing among rootstock treatments averaged across scion treatments indicating these traits were unaffected by the rootstock treatments. Combinations of high or low leaflet orientation with normal or prolific rooting morphology had no significant ($p \leq 0.05$) discernable effect on whole plant transpiration. The high leaflet orienting line scion treatment, USG 5601T, had higher yield and used less water per unit yield than the low leaflet orienting line scion treatment, PI 416937. Although this is anecdotal due to the genetic differences of the lines per se, it may lend some support to the idea that plants with high leaflet orientation are adapted to have increased yields with better water use efficiencies. The lack of effect of the PI 416937 prolific rootstock on whole plant transpiration across scion treatments supports findings that the differential slow wilting and transpirational attributes of this line may not be as related to the root morphology as previously speculated. Since there was abundant soil moisture through the 2003 growing season, it is not known whether the leaflet orientation or prolific rooting traits would have been beneficial during a moisture stressed environment.

Population line development and evaluation

The experiments associated with the population line development and evaluations were conducted across the state of Tennessee (USA) during the 2005, 2006, 2007, and 2008 growing seasons. Two hundred and ten $F_{4:6}$, $F_{7:8}$, $F_{7:9}$, and $F_{7:10}$ lines from the cross USG 5601T × PI 416937, which segregated for the two traits of interest, were evaluated for whole plant transpiration rates, single plant yield, biomass production, leaf area, seed size, leaflet transpiration, stomatal conductance, photosynthesis rates, photosynthetically active radiation (PAR), solar radiation (SAR), leaflet temperatures, leaflet orientation and root morphology scores at Knoxville, TN USA (35.89 lat., -83.96 long.). Whole plant transpiration was measured on two to four plants of each line in each year of the study using a Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stages of growth (R4-R6). The amount of water transpired by the treatment in a 24 h period during seed fill was divided by the grams of seed produced by that plant in order to obtain estimates of water use efficiency. Leaflet temperatures were measured with a Raytek model ST20 Pro infrared thermometer (Raytek Corp., Santa Cruz, CA). PAR measurements were taken with a model SF40 Decagon Sunflec Ceptometer (Decagon Devices Inc., Pullman, WA). Data for leaflet transpiration, stomatal conductance, and photosynthesis rates were obtained using the Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX). Protein and oil analysis was performed on a Foss Model 1229 NIR analyzer (Foss NIRSystems Inc., Laurel, MD). Replicated plots were also planted at Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan, TN (35.93 lat., -88.70 long.). All data were analyzed using SAS Proc

Mixed with the soybean lines considered as fixed effects and all other effects considered random in order to obtain least squares means of traits for each line for each year/location. Least squares means of each line were then used in the correlation and phenotypic class analyses (SAS User Guide 9.1.3, 2006). High leaflet orienting parental line, USG5601T, exhibited lower mid-canopy reductions in rates of leaflet PAR, temperature, transpiration, stomatal conductance, and photosynthesis than the low leaflet orienting parental line, PI 416937. Frequency distributions of population lines for leaflet orientation and root morphology scores approximated normal distributions suggesting that the two traits are polygenic in nature. Light penetration into middle canopy was significantly higher for population lines which exhibited high leaflet orientation than for those that exhibited medium or low leaflet orientation. Leaflet temperatures of high leaflet orienting population lines averaged 5.2°C cooler than leaflets exposed to full direct sunlight. Low leaflet orienting lines in this population were associated with better water use efficiency ($r=-0.28$, $p=0.04$), later maturity ($r=0.34$, $p=0.01$), larger seed size ($r=0.30$, $p=0.03$), higher leaf area ($r=0.42$, $p=0.002$), lower seed oil ($r=-0.47$, $p=0.0003$), higher seed protein ($r=0.18$, $p=0.01$), and higher biomass accumulation ($r=0.42$, $p=0.002$). There were no significant associations between lower leaflet orientation and whole plant transpiration or yield, however, there were patterns that suggested a general relationship between higher transpiration and higher yield. High leaflet orienting lines were associated with the same traits but in the opposite manner. Many of these phenotypic trait associations are consistent with the parental phenotype suggesting some degree of linkage. Leaflet orientation and root morphology were found to be significantly correlated ($r=0.33$, $p=0.02$). Population lines exhibiting prolific rooting were associated with higher transpiration rates ($r=0.29$, $p=0.03$), higher yield ($r=0.29$, $p=0.03$), later maturity ($r=0.49$, $p=0.0002$), lower seed oil ($r=-0.28$, $p=0.04$), higher leaf area ($r=0.43$, $p=0.001$), higher biomass accumulation (r=0.37, p=0.006), and higher upper canopy leaflet photosynthesis rates (r=0.49, p=0.0002). A single correlation between prolific rooting and better water use efficiency (r=-0.33, p=0.01) was detected however the strength of the data was not compelling as not all associations were significant or in the same direction in the correlation and phenotypic class analyses. Normal rooted lines were associated with the same traits but in the opposite manner. Many of these phenotypic trait associations were consistent with the parental phenotype again suggesting some degree of linkage. No significant differences were detected between the combination classes which represent the extreme phenotypic leaflet orientation and root morphology combinations of H/N, L/P, H/P, and L/N for whole plant transpiration, single plant yield, plot yield, water use efficiency, or seed protein content indicating that no additive gain was realized. This lack of an additive or reductive response could be due to the correlation between leaflet orientation and root morphology which was detected in this study. Regarding associations between the leaflet orientation and root morphology traits and whole plant transpiration and yield, it is likely that leaflet orientation (rather than root morphology) is responsible for detected differences since other studies involving grafted plants and isogenic pairs found no effect on these traits when comparing normal roots to that of prolific roots. Significant differences were detected in some single year analyses between the combination classes which represent the extreme phenotypic combinations of H/N, L/P, H/P, and L/N for seed size, maturity, plant height, lodging, seed oil content, dry weight biomass accumulation, and leaf area. However, the patterns observed only reflected the previously described trends of lower leaflet orientation being associated with larger seed size, later maturity, slightly increased plant height and lodging, lower seed oil content, higher biomass accumulation, and higher leaf area. The root morphology phenotypes seemed to have little to no effect on the expression of these traits when analyzed as combined phenotypic classes.

Near-isogenic line pair development and evaluations

The near-isogenic line development and evaluation experiments were conducted across the state of Tennessee (USA) during the 2006 and 2007 growing seasons using $F_{3:6}$, $F_{3:7}$, $F_{4:7}$, and $F_{4:8}$ near- isogenic line pairs. Growing conditions across the experimental locations in 2006 were characterized by abundant soil moisture while the 2007 growing season sustained record drought conditions. Whole plant transpiration rates, single plant yield, biomass production, leaf area, seed size, leaflet transpiration, stomatal conductance, photosynthesis rates, photosynthetically active radiation (PAR), solar radiation (SAR), soil moisture, leaflet orientation and root morphology scores were measured at Knoxville, TN USA (35.89 lat., -83.96 long.) on several successive days. Whole plant transpiration was measured on four plants of each line in each year of the study using the Dynamax Flow 32 Sap Flow Monitoring System (Dynamax Inc., Houston, TX) when the plants were in the active pod filling stage of growth (R4-R6). The amount of water transpired by the treatment in a 24 hour period during seed fill was divided by the grams of seed produced by that plant in order to obtain estimates of water use efficiency. In order to evaluate yield and other agronomic traits, replicated plots were also planted at Knoxville, Springfield (36.48 lat., -86.82 long.), Spring Hill (35.72 lat., -86.96 long.) and Milan, TN (35.93 lat., -88.70 long.). Leaflet transpiration, stomatal conductance, and photosynthesis rates data were obtained using the Dynamax LCi Photosynthesis meter (Dynamax Inc., Houston, TX). Protein and oil analysis was performed on a Foss Model 1229 NIR analyzer (Foss NIRSystems Inc., Laurel, MD). All data were analyzed using SAS Proc Mixed with the soybean lines considered as fixed effects and all other effects considered random in order to obtain least squares means of traits for each line for each year and location. Least squares means of all lines were then used in the correlation and phenotypic class analyses (SAS User Guide 9.1.3, 2006). The current study detected no consistent patterns or significant effects due to differing leaflet orientation and root morphology scores among this set of near-isogenic lines for any of the measured traits. The current study was limited by the lack of prominent differences in leaflet orientation and root morphology between the near-isogenic line pairs. It is somewhat probable that this lack of more prominent differences affected the results of this study. Further study is needed to determine the effects of leaflet orientation and root morphology on whole plant transpiration, yield, water use efficiencies, and other agronomic characteristics in soybeans.

Restrained canopy leaflet evaluations

The objective of this study was to evaluate the effects of natural and artificially imposed leaflet orientation on transpiration rates and other physiological traits in soybeans. The soybean cultivar USG 5601T was chosen for this study due to its ability to strongly orient its leaves during the day in response to sunlight. Twenty-four plants were subjected to two treatments during the 2007 and 2008 growing season in Knoxville, TN. One treatment set was restrained with netting in order to gently force the orientation of the outer canopy leaves to assume the phenotype of a plant which does not orient its leaves. The other treatment was unrestrained and allowed to orient its leaves as normal. Whole plant transpiration rates of 24 plants for each treatment were measured for a 24 h period with the Dynamax Flow 32 Sap Flow instrument when the plants were in the R5 growth stage of active pod filling. Leaflet temperatures were measured with a Raytek Infrared Thermometer. Photosynthetically Active Radiation (PAR) measurements above and mid-canopy were taken with a Decagon Sunflec Ceptometer. There were no statistical differences found between the average transpiration rates of unrestrained plants, which oriented their leaves and restrained plants which were not allowed to orient their leaves. Temperatures of leaflets which were restrained from movement were 3.3°C higher in temperature than leaflets which were allowed to orient in a paraheliotropic manner. Plants which were unrestrained had significantly higher levels of PAR in their mid-canopy area than plants which were restrained. It was speculated that the higher leaflet temperature of the restrained plants would result in higher overall transpiration rates than unrestrained plants. The high leaflet orientation and associated lower leaflet temperatures of the unrestrained plants may have resulted in a lower transpiration rate for upper canopy leaflets as other studies have found previously. However, the unrestrained soybean plants with high leaflet orientation allowed more sunlight into the lower canopy which may have resulted in higher rates of transpiration and photosynthesis for those leaves relative to the restrained plants with lower leaflet orientation. This may account for the consistent but non-significant 24.4 g d^{-1} increase in whole plant transpiration by the higher leaflet orienting unrestrained plants.

Studies related to this research and suggested studies for further research

Seed from the 2003 and 2004 grafting studies were used to investigate the contributions of differing scions and rootstocks to concentrations of isoflavone, protein, oil, and amino acids. It was determined that the scion conditioned the concentrations of these seed constituents while the rootstock had little to no effect. Hydraulic conductivity research regarding phenotypic classes of soybeans differing in leaflet orientation and root morphology was conducted in 2007 and 2008 using a Dynamax High Pressure Flow Meter (Dynamax, Inc, Houston, TX). No differences were detected among the differing phenotypic classes for hydraulic conductivity. Data were collected during this project which can be used to calculate both broad and narrow sense heritability estimates via entry means analyses and parent-offspring regression analyses. Soil moisture probes were placed in the root zones of soybean population lines which differed in leaflet orientation and root morphology in the 2005, 2006, 2007, and 2008 growing seasons. This data can be used to investigate soil moisture depletion differences which may be related to differing leaflet orientation and root morphology phenotypic classes. A data set from a local weather monitoring station was discovered which recorded several types of environmental data on 30 minute intervals concurrent with the whole plant transpiration data recorded in the current study in the years 2003, 2005, 2006, 2007 and 2008. These two data sets can be combined and analyzed for further investigation of relationships between transpiration and environmental factors. A recombinant inbred line population (USG 5601T × PI 416937) consisting of 956 lines was developed by modified single seed decent. This population is currently in the F8 generation and may be released to allow other researchers the opportunity to study the many contrasting and segregating traits brought together in this population from the parents.

Additional research is suggested to further investigate the effects of leaflet orientation and root morphology on water use and yield in soybeans. The grafting study should be conducted again in order to get a second year of experimental data. Increased access and use of photosynthesis meters would be very useful in investigating differences in transpiration and photosynthesis of leaflets at different canopy levels among soybean lines differing in leaflet orientation and root morpholgy. Development of superior near-isogenic line pairs could be attempted by selecting larger numbers of single plants from advanced generation segregating lines. Selection of segregating lines in the F4, F5, F6 geneations along with the advancement and evaluation of 50 – 80 single plant harvested progeny rows, might result in detection and development of near-isogenic pairs with more prominent phenotypic differences than were found in the current study. Since leaflet orientation and root morphology seemed to be linked in this study, it is suggested that these traits be studied separately by population development and analysis. The population of the current study contains lines which would allow crosses to be made which would likely segregate for leaflet orientation or root morphology alone. Two normal rooted lines which differed in leaflet orientation could be crossed to produce a population which only segregates for the leaflet orientation trait. Similar phenotypic crosses could be made which would produce populations that would maintain one condition of the root or leaflet phenotype while allowing the other trait to segregate. This would allow a more detatiled study of the traits individually without the potential confounding due to the observed correlation. The lines developed in the current study were never subjected to moderate or severe drought conditions. It is therefore unknown what the effects of the differing leaflet orientation and root morphology phenotypic classes may have been under drought stress. Future studies should ensure that lines with these traits be subjected to drought stress and measured for effects. Attempts were made in the current study to provide this type of data by planting selected lines in two drought prone regions - the Sand Hills area of North Carolina and Stuttgart, Arkansas. One location suffered severe drought coupled with irrigation equipment failure which resulted in a loss of all data. The other location received abundant rainfall and was therefore not useful as a drought stress environment. The 956 line recombinant inbred population which was developed as part of this project offers the opportunity for further phenotyping and molecular marker studies. It is the authors hope that the data and genetic material generated by this project will prove useful for further studies.

Vita

The author, Richard DeWayne Johnson, was born in Lawrenceburg, Tennessee in 1966. He was raised by his paternal grandparents on a small farm in rural Lawrence County, Tennessee where he learned about the value of traditional, old-style farming, manual labor and animal based agriculture where mules and hoes were the main tools of the trade.

He attended elementary schools in Tennessee, Nevada, California, and Alabama. He graduated from Loretto High School, Loretto, Tennessee, in 1984 where he was very active in the Future Farmers of America. Upon graduation, he enrolled in the school of life and work, farming and working in the local casket factory. In 1987, he enrolled at Columbia State Community College and graduated summa cum laude with an Associate of Science degree in Agriculture two years later. He then transferred to The University of Tennessee at Martin and in 1991 graduated summa cum laude with a Bachelor of Science degree in Agriculture. In May 1991, he entered graduate school at Purdue University and worked in the area of sorghum breeding and genetics. In 1993, he transferred to the University of Tennessee accepting a position with the Plant and Soil Science Department as a Senior Research Associate in the Soybean Breeding and Genetics program. After earning his Master of Science degree in Agriculture in 1997, he occupied positions in the seed industry during a time of rapid company acquisitions and change. During this time he worked as a research associate in transgenic corn conversion in Hawaii, an assistant soybean research station manager / breeder in Ohio, and an interim corn research station manager / breeder in Indiana. In 2002 he returned to the University of Tennessee accepting the position as research associate in the Variety Testing Program.